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HISTOLOGY OF ADENOMA OF THE ISLETS OF LANGERHANS

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In 1924, Seale Harris [1] presented evidence to indicate that spontaneous hyperinsulinism might result from overactivity of the islet cell tissue of the pancreas. More recently, it has been shown by several authors that comparable manifestations are due to the endocrine activity of islet cell neoplasms. Five of these tumors were removed in the surgical clinics of Washington University following preoperative diagnoses of chronic hypoglycemia; in all, the subsequent history of the patients proved the tumor to have been the actual cause of the symptoms. Detailed pathologic reports on three of the five tumors accompanied the case reports of Carr, Parker, Grove, Fisher and Larimore,[2] Womack, Gnagi and Graham,[3] and Graham and Womack.[4] Two of the cases are as yet unreported.

The present communication concerns the results of a comparative histologic study of these five tumors; more particularly, we have been interested in (1) genesis, (2) the appearance of the cells of each as compared with normal human islet cells, (3) the stage of development and (4) the effect of tumor secretion on the normal islets of the pancreas in which the tumor was found. The basis of such a study has been amply provided by past investigators, since accurate knowledge concerning the islets of the pancreas is not exceeded by that relating to any other endocrine gland.

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1. Harris, S.: J. A. M. A. **83**:729, 1924.

2. Carr, A. D.; Parker, R.; Grove, E.; Fisher, A. O., and Larimore, J. W.: J. A. M. A. **96**:1363, 1931.

3. Womack, N. A.; Gnagi, W. B., and Graham, E. A.: J. A. M. A. **97**:831, 1931.

4. Graham, E. A., and Womack, N. A.: Surg., Gynec. & Obst. **56**:728, 1933.

PROCEDURE

The tumors were available for fresh study and for fixation immediately following their removal. The procedure did not vary. Following a brief inspection, the tumor was sliced in a sagittal plane and the segments were fixed in various solutions.[5] Bits were placed in Ringer's solution and in 1:2,000 neutral red in physiologic solution of sodium chloride for fresh examination. The position and gross characteristics of the pieces placed in the various fixatives and of the fresh specimens were noted.

In each case, portions of adjoining pancreas were adherent to the tumor. These were fixed in the same fluids and subsequently dehydrated, embedded, cut and stained with the tumor material.

For staining, we relied on hematoxylin and eosin, the Mallory azan stain, the neutral dyes of Bowie and Bensley, which color the individual granules of appropriately fixed islet cells, and aniline acid fuchsin followed by Mallory II stain. In several cases, groups of islet cells were identified following one method of staining and were drawn with the camera lucida; the slide was then destained and colored again with a different stain. This procedure served to identify and locate the characteristic coloring displayed by the specific granules of each cell with several methods; it was done for both normal islet and tumor tissue.

The bits for fresh study were utilized as rapidly as possible, usually within ten minutes following removal of the tumor. They were placed on slides in a drop of Ringer's solution and teased to particulate form, and a coverslip was applied. After a brief survey with the low power microscope, typical tumor cells were studied under the oil immersion lens and their characteristics noted. The material immersed in neutral red was similarly treated and studied.

PANCREATIC ADENOMAS PRODUCING HYPOGLYCEMIC SYMPTOMS

The clinical histories of the five patients whose tumors are hereafter subjected to comparative study are given in case reports by Carr and his co-workers,[2] Womack and his co-workers,[3] and Graham and Womack.[4] For convenient reference, the essential facts are summarized in the table. Here and in the subsequent observations, the tumors (and cases) are designated by Roman numerals. In case III, two tumors were removed. The removal of *a*, a nonspecific mass, did not alleviate the hypoglycemic symptoms; at a second operation, *b*, a true adenoma, was removed with successful results.

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5. These comprised: aqueous chrome sublimate (potassium dichromate, 2.5 Gm.; mercuric chloride, 5 Gm.; distilled water, 100 cc.), modifications of the same, namely, (1) acetic chrome sublimate (5 cc. of glacial acetic acid and 95 cc. of aqueous chrome sublimate), and (2) formaldehyde chrome sublimate (10 cc. of formaldehyde and 90 cc. of aqueous chrome sublimate), Bouin's fluid (saturated aqueous solution of trinitrophenol, 75 cc.; formaldehyde, 25 cc.; glacial acetic acid, 5 cc.), Altmann's fluid (equal parts of a 5 per cent solution of potassium dichromate and a 2 per cent solution of osmic acid) and a diluted solution of formaldehyde, U. S. P. (1:10) in 90 per cent alcohol. Pieces of one large tumor were also fixed in Regaud's fluid (8 cc. of a 3 per cent solution of potassium dichromate and 2 cc. of formaldehyde) and in Bensley's acetic osmic dichromate fluid (8 cc. of 2.5 per cent solution of potassium dichromate, 2 cc. of a 2 per cent solution of osmic acid and glacial acetic acid, 1 drop).

1. *Gross Characteristics of the Tumors.*—The five tumors removed varied in size, consistency, weight and color. Tumors I and II were approximately 1 by 2 cm. in size, purple-red and friable. Tumor III*a* was ovoid, of similar color, unyielding to the touch and 1 by 0.8 cm. in size. Tumor III*b* was somewhat larger than those previously considered (2 by 1.4 by 1.2 cm.), ovoid, yellowish gray and of firm consistency. Tumor IV was small, 1.5 by 0.8 cm., and similar in color to III*b*. Tumor V was also yellowish gray and of soft consistency. The surface was nodular and in some areas felt cystic; several lesser masses were suspended from the tumor by pedicles. It was extremely large for tumors of this type (9 by 9 by 11 cm.; weight, 500 Gm.).

The internal configuration also varied. The cut surface of tumors I and II presented an appearance similar to that noted externally. Tumor

Summary of Clinical Data Relating to the Tumors

Tumor No.	Sex	Age	Duration of Symptoms	Severity at Time of Operation	Postoperative History
I	M	19	1 year	++	Complete absence of hypoglycemic seizures
II	M	44	2 years	++	Same as tumor I
III	M	27	3 years	A. ++++ B. ++++	Temporary relief Same as tumor I
IV	F	44	4 years	+	Same as tumor I
V	M	52	2 years	+++	Same as tumor I

III*a* had a calcified core surrounded by purple-red friable tissue. Tumor III*b* resembled a cut fibroma; embedded in the capsule was a single nodule of purple-red friable tissue, 3 mm. in diameter. Tumor IV had a necrotic central core; tumor V, a calcified core with projections toward the surface. With the exception of the calcified core, tumor V consisted of a solid mass of grayish-yellow, soft, friable tissue; no cysts were found.

The tumors differed in degree of encapsulation. Tumors I and V were well encapsulated, tumor II was not encapsulated and tumors III*a* and *b* and IV were partially encapsulated. Further details with regard to encapsulation are given with the description of the architecture of the tumor.

2. *Study of Fresh Cells Immersed in Ringer's Solution.*—(*a*) Normal Islets: The researches of Laguesse [6] and Bensley [7] showed that the appearances of the cell which serve to identify islet tissue in fixed and stained preparations are also obvious in isolated fresh cells immersed in an isotonic saline solution. Such cells, studied without fixation, nearly

6. Laguesse, E.: Revue générale d'histologie, Paris, A. Storck & Cie, 1908, vols. 2 and 3.

7. Bensley, R. R.: Am. J. Anat. **12**:297, 1911.

approximate the living condition; living islet cells with blood supply intact may be studied in the pancreas of the anesthetized mouse drawn out on a microscope stage between slide and coverslip (Covell,[8] O'Leary [9]).

Bensley's [7] detailed description of the fresh, isolated islet cells of the pancreas of a guinea-pig serves as an adequate norm for comparison with islet cells isolated from the adenomas studied. Abstractly, comparison of the normal islet cells of one species with the tumor islet cells of another may not seem justifiable, but actually, any differences which may exist among normal cells of different species are too slight for even the most expert to detect. A summary of our own experience in the study of isolated cells of normal islets of the mouse, guinea-pig and one child of 1½ years follows:

The fresh unstained cells of normal islets are polymorphic, most frequently polyhedral or columnar. Cell boundaries are usually distinct. Embedded in the cytoplasm, and more refractile, are tiny granules of uniform size, densely packed. This contiguity of granules throughout the cytoplasm is interrupted only by occasional lipoid droplets, small vacuoles and a series of granule-free canals identical with the apparatus of Golgi, which may be identified in fixed tissue after staining with special osmic acid or by the reduced silver method. The nucleus, ovoid or spherical, appears homogeneous unless intense illumination is used; then the nucleolus and lesser lumps of chromatin are apparent.

Cells of normal islets in the mouse and guinea-pig may also be subjected to *supra* or *intra vitam* staining. Supravitally, neutral red or janus green B (dilution 1 : 10,000) is perfused through the aorta of an exsanguinated guinea-pig. Intravitally, neutral red is injected intravenously or subcutaneously (from 0.1 to 0.5 cc. of a 1 per cent solution); janus green B, by dripping a 1 :1,000 dilution on the exposed living pancreas.

By both *intra* and *supra vitam* methods, the end-results of staining with neutral red and janus green B are the same. The specific granules of all islet cells which come in contact with the dye are colored sharply. With neutral red, the lipoid droplets of the fresh cells also stain; with janus green B, the slender filamentous mitochondria are sometimes colored, as well as the specific granules.

(*b*) Adenomas: In tumors I, II and V, the cells were readily isolated for fresh study; in III*b* and IV, with difficulty.

In all tumors, except III*a*, isolated islet cells were obtained which were practically identical with the previously described unstained living cells of normal islets. The haze of tiny granules studded the entire

8. Covell, W. P.: Anat. Rec. **40**:213, 1928.
9. O'Leary, J. L.: Anat. Rec. **45**:27, 1930.

cytoplasmic mass, with the exception of the areas occupied by the canals of Holmgren, occasional lipoid droplets and small vacuoles. The nucleus was large and vesicular. Occasional lumps of chromatin and nucleoli were observed. In tumor V, cells also occurred in which the granules varied considerably in size and did not occupy the whole mass of cytoplasm. Cells with large nuclei, rich in chromatin, were frequent as were also binucleated cells and giant cells with several nuclei.

The abundance of material available for study in tumor V made it possible to set aside a piece of tissue immersed in cold Ringer's solution for twenty-four hours. At the end of this period, many cells had not altered appreciably from the appearance observed shortly following the removal of the tumor. This indicates that the tumor islet cells are very resistant to postmortem change. Isolated normal islet cells of the guinea-pig, in contrast, sometimes undergo marked deteriorative changes one-half hour after removal from the body (Bensley [7]).

It has been constantly observed in the study of tumors (first by R. R. Bensley, later by ourselves) that when neutral red is applied in 1:2,000 dilution in physiologic solution of sodium chloride, the specific granules of only a minority of the islet cells color with the dye. That the failure of the granules of the majority of the cells to color (like the cells of normal islets) is not due to mechanical difficulties is readily demonstrable (1) by the occurrence of cells with unstained granules besides other cells with colored granules, and (2) by the fact that if a piece of pancreas of a guinea-pig is immersed in neutral red simultaneously with the bit of tumor, the granules of all cells of the islets of the guinea-pig will be stained, while only those of the minority of the tumor cells are colored. The granules of the majority and of the minority of cells, therefore, differ in their affinity for neutral red, indicating a difference in the composition of the granules. In tumor V, the cells with colored granules were more numerous than in any of the other tumors studied; the granules varied somewhat in size, and in many cells those stained by neutral red and the unstained granules could be observed side by side in the cytoplasm.

The content of cells revealed by fresh study in tumor III*a* and the pocket of purple-red friable tissue in tumor III*b* will be considered separately because of the dissimilarity of the cells to those which were the principal components of tumors I, II, III*b*, IV and V. The majority of cells were macrophages, lymphocytes and fibroblasts. A minority of mast cells and polymorphonuclear leukocytes were also observed. Macrophages and lymphocytes were also seen in significant numbers during the fresh study of the unquestioned islet cells tumors.

3. *Architecture of Normal Islets as Compared with That of the Adenomas.*—(*a*) Normal Islets: The characteristics which serve to distinguish the islets of the pancreas from duct and acinar tissue in the

usual hematoxylin-eosin preparation are so well known that comment is unnecessary. However, the architectural relations of cells, capillaries and connective tissue framework must be understood, since it is apparent that in the formation of islet cell adenomas the tendency will be toward a repetition of these relations on a large scale.

The cells of the islet (mouse) form anastomosing cords, separated by capillary channels, which pursue a course varying from straight to very tortuous through the islets. Some capillaries traverse islet tissue in passing between one acinar lobule and another; others appear to originate from short arterioles. Frequently, venous channels extend directly from an islet to a neighboring vein. A comprehensive account of the circulatory pattern of the islets is given by Beck and Berg.[10]

The cell-vascular relationship is an intimate one, and two or more surfaces of a single cell may abut on the capillaries. However, the contact is indirect, since a definite endothelial membrane is usually interposed between the islet cells and the vascular channels. In occasional capillaries, this membrane is not demonstrable, but the possibility is not excluded that these may be lymphatics.

The islets are separated from the acinar tissue by a partition of connective tissue of variable thickness which appears to be more closely adherent to the surfaces of the acinar lobules than to the islet cells. In the interstitial islets, which lie free from acinar investment along the larger blood vessels, this peri-insular framework is strengthened. Reticular tendrils about the capillaries are the only projections of connective tissue into the interior of the islets.

(*b*) Adenomas: Tumor III*b*, a young, growing adenoma with excellent accommodation between the parenchymatous and the vascular elements, serves as a starting point from which to develop the architectural characteristics of the group. Study at a low magnification of a section through the center of the tumor stained with Mallory azan stain shows an interlacing stroma of collagenic tissue, a central core containing blood vessels, ducts and ducts differentiating into islet tissue and a broken capsule surrounding the tumor. The parenchyma is divided into sinuous cords and lobules of islet cells, one or two (rarely more) cell layers in thickness, separated by the stroma and by arterioles and capillaries which issue from it (fig. 1 *A*). It is to be noted that the capillaries are thin-walled, very similar in appearance to those which pervade normal islets; the only connective tissue elements usually found about them are reticular fibrils. In areas adjacent to the acinar tissue of the pancreas, the connective tissue is richer and has separated the parenchyma into islands served by the capillary vessels of penetrating arterioles. Hyalinization about, and thickening of, the walls of the smaller vessels are so seldom seen that these changes are negligible. It is apparent, therefore, that this tumor is representative of Bensley's stage I in the chronological classification of islet cell adenomas (Womack and his co-workers[3]).

10. Beck, J. S. P., and Berg, B. N.: Am. J. Path. **7**:31, 1931.

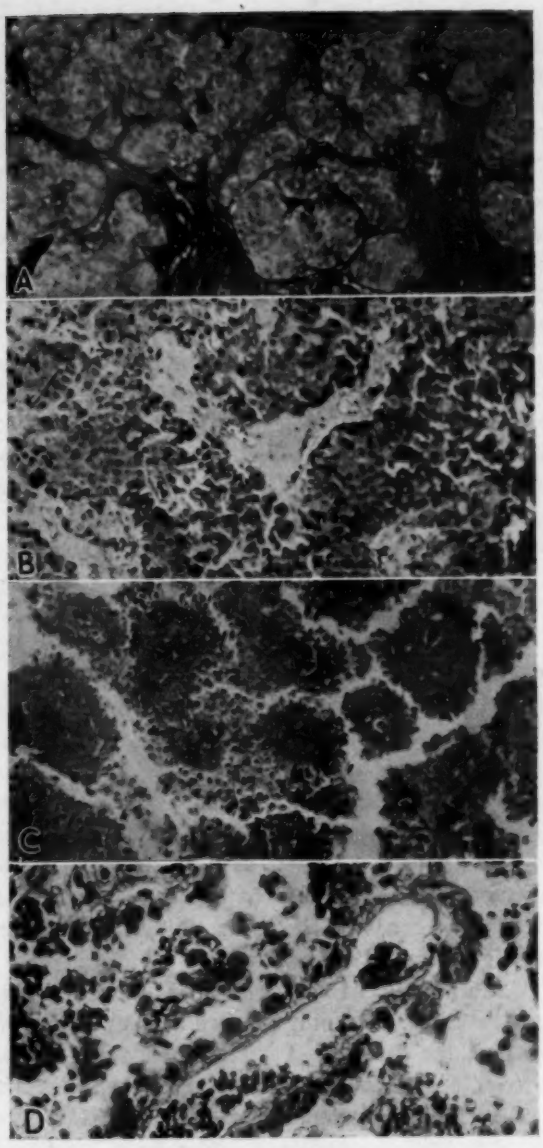

Fig. 1.—*A*, low power photomicrograph, typical area, tumor III*b*. This represents the youngest stage of tumor development observed in the series. Note the intimate relationship of cells and capillaries. *B*, from tumor V, illustrating a less perfectly organized architecture than that observed in *A*. Note the hyalinization in the vessel walls and very much less intimate cell-capillary relationship. *C*, from tumor II. The latest stage in tumor architecture observed. The vessels are severely hyalinized, and tumor cells cling to their surfaces. The intervening spaces contain blood and the débris of necrotic cells. *D*, thin-walled vascular channel from tumor V surrounded by a sheath of tumor cells. Note the looseness of the surrounding area. All photomicrographs except *D* are enlarged to 160 diameters; *D* is enlarged to 240 diameters. Zeiss 16 mm.; ocular Homal I.

Large areas of tumors IV and V are representative of a later, more disorganized stage of growth. The parenchymatous elements have increased out of all proportion to the vascular bed, resulting in the formation of thick cords and balls of cells, the center of which may be separated from the capillary vessels by three or four layers of cells (fig. 1 *B*, tumor V). Coincidentally, the majority of the small vessels have accumulated collagenic connective tissue, or their walls have thickened and the lumens become occluded by hyalinization. The latter tendency is most manifest in tumor V.

The result of this more disorganized growth has been the focal degeneration of cell groups, leaving cavities containing cellular débris and blood elements. Groups of cells with diffuse basophilic staining of the cytoplasm and pyknotic nuclei were also observed. We have been unable to find convincing evidence that these manifestations of degeneration are entirely attributable to a poor vascular supply, since they sometimes occur in the neighborhood of apparently functioning vessels. One area in tumor V suggests the end-result of this degeneration of islet tissue. Here the ghosts of former vessels, occasional functioning ones and a few masses of apparently normal islet cells occur in a dense collagenic stroma.

Although the vascular supply of the parenchymatous elements in each of tumors IV and V was not adequate as a whole, free corpuscles were sometimes observed between normal cells, and occasionally an endothelial nucleus was interposed between these and the surrounding islet cells. This may represent a belated attempt toward more efficient vascularization. Also, in small areas of both tumors IV and V, conditions reminiscent of the major area of tumor III*b* were found. Here, two and three layered cords of islet cells abutted directly on capillaries with thin walls free from hyalinization.

In tumor V, an area was found which was not duplicated in any of the other tumors studied. The only marked accumulations of connective tissue accompanied the arteries. Peculiar to it was the occurrence of numerous thin-walled vascular tubes having a single layered or multilayered investment of parenchymatous cells (fig. 1 *D*). Sometimes edematous connective tissue intervened between the outer surfaces of the tubes and the tumor cells; mostly the cells were attached directly to the walls of the vessels. Tumor cells were infrequently located in these thin tubes and also in the larger vessels of the area. Between the cell-coated tubes there were cellular débris and recently extravasated red blood corpuscles.

Tumors I and II were characterized by the occurrence of areas of a later stage than that considered most typical of tumors IV and V. Tumor I had predominantly this arrangement; tumor II, in part. The appearance was that of anastomosing cords of hyalinized connective

tissue separated by cavities containing extravasated blood and necrotic remnants of tumor cells (fig. 1 *C*). Clinging to the walls of the cavities were single or multiple layers of islet tumor cells which varied in appearance; some were obviously degenerated, and many appeared to be quite healthy.

4. *Characteristics of Normal Human Islet Cells Compared with Those of Adenomas.*—(*a*) Normal Islet Cells: The salient characteristics of islet cells have been noted in describing their appearance in the living, unstained state. Since the fixed tissue equivalents of the cell components do not differ in material detail, we may next consider the basis of classification into cell types.

Following appropriate methods of fixation and staining, three cell types can be identified in the islets of Langerhans of the human pancreas. Two of these, alpha and beta, were first defined by Lane [11] in the pancreas of the guinea-pig. Lane observed that following fixation in 70 per cent alcohol, the specific granules of the alpha cells stained purple with neutral gentian; in the remaining (majority) cells of the islets the granules had dissolved and the cytoplasm remained clear. Conversely, following fixation in chrome sublimate and staining with neutral gentian, the specific granules of the second cell type (called beta by Lane) colored; those of the alpha variety did not color. Bensley [7] later successfully stained the specific granules of the alpha and beta cells differentially in the same islet with aniline acid fuchsin and methyl green, following fixation in his own acetic-osmic bichromate fluid. Bloom [12] was able in human islets, following fixation in formaldehyde chrome sublimate, to stain these two cell types and a third (D) by using the Mallory azan stain. Henceforth, the three cell types will be referred to as A, B and D.

Experimental studies (Allen [13] and Homans [14]) designed to effect exhaustion of the cell type of the islets, active in the production of insulin, through overwork of a greatly reduced pancreas resulted in hydropic degeneration of the B cells; the A variety remained normal. Restoration of the normal appearance of the exhausted B cells of such overworked islets has also been brought about by the administration of proper doses of insulin (Copp and Barclay [15]). Control pieces of pancreas, taken before the institution of insulin therapy, showed the B cells to be in an advanced state of hydropic degeneration. These data, together with inferences concerning the relative alcohol solubility of the granules of the A and B cells, indicate that the B variety is active in the production of insulin.

The three cell types occur constantly in human islets. Bloom finds that the B cells are much more numerous; A and D cells occur in about

11. Lane, M. A.: Am. J. Anat. **7**:409, 1907.
12. Bloom, W.: Anat. Rec. **49**:363, 1931.
13. Allen, F. M.: J. Metab. Research **1**:1, 1922.
14. Homans, J.: J. M. Research **30**:49, 1914.
15. Copp, E. F. F., and Barclay, A. J.: J. Metab. Research **1**:445, 1923.

equal numbers. In our material the average frequency of occurrence is in the order B, A and D.

Material fixed in formaldehyde chrome sublimate was used for all of our comparisons between the staining reactions of the specific granules of normal and tumor islet cells. After staining with Mallory azan stain, normal islet A cell granules colored deep red; B cell granules, lilac to gray-yellow; D cell granules, light blue. Bowie's [16] neutral stain colors the granules of the normal islet B cells deep blue; [17] those of A and D cells, light blue, pink, or not at all. Normal islet B granules color purple; A and D granules do not color with Bensley's neutral gentian. However, when a section is stained by Bowie's or Bensley's neutral dyes and destained and Mallory azan stain is applied, it is apparent that some overlap occurs. Some of the cells designated as A cells in preparations stained with Mallory azan stain may appear to be B cells after being stained with Bowie's dye. This discrepancy suggests that those comparisons which necessitate assigning types to islet cells be made by the Lane [11] method.

Differential staining of the specific granules by the Lane method is the one good criterion for assigning types to islet cells. Other cell characteristics, which have been said to differ from one type to another, are the shape and chromatin content of the nucleus, sharpness of cell outline, granule density and position of the cells relative to the capillaries. However, in nonspecifically stained preparations (man) these characteristics are hardly sufficient to distinguish one type from another.

Islet cells give a negative reaction for enterochromaffin (argentaffin) granules with Masson's method.[18] This is of importance since the argentaffin cells of the gastro-intestinal epithelium may also form tumors. The type enterochromaffin cell is cylindric or flask-shaped; the basal portion of the cytoplasm (between the nucleus and the capillary pole) is studded with granules in size similar to the specific granules of the islet cells. In some animals, though not usually in man, these are more uniformly distributed throughout the cytoplasm. The granules reduce silver by the specific method employed by Masson for material fixed in Bouin's fluid; the specific granules of the islet cells do not. Van Campenhaut,[19] using a block method of silver impregnation, recently succeeded in demonstrating that the specific granules of certain of the islet cells, as well as enterochromaffin granules, reduce silver. None of our tumors or normal pancreases were so prepared.

(*b*) Tumor Islet Cells: Just as the parenchymatovascular accommodation in tumor III*b* appeared the most perfect in our series, so the great majority of tumor cells more closely approximated the appearance

16. Bowie, D. J.: Anat. Rec. **29**:57, 1924.
17. Bensley: Personal communication to the authors.
18. Masson, P.: Am. J. Path. **4**:181, 1928.
19. Van Campenhaut, E.: Proc. Soc. Exper. Biol. & Med. **30**:617, 1933.

of those of normal islets than in any of the other adenomas studied. The healthy appearing cells, of which this tumor had a large majority, presented a slender columnar, flask-like or polyhedral appearance, depending on their position in the sinuous cords and lobules (fig. 2 *A*). The nuclei were similar in size and chromatin content to those of normal islet cells. Most often the cytoplasm appeared to be evenly studded with specific granules, with the exception of the areas occupied by the canals of Holmgren and cytoplasmic inclusions (p. 294); sometimes they were concentrated at the capillary poles. In tumors I and II, the healthiest appearing cells were very similar to those just described. In tumors IV and V, polyhedral or rounded cells predominated. The nuclei varied in size from normal to one-half again as large, and also in chromatin content. The specific granules were evenly distributed in many cells; in others they were concentrated about the nucleus, leaving a granule-free zone at the periphery. No significant difference in size of the granules of a single cell was apparent. Binucleated cells and giant cells with central clusters of nuclei were not uncommon.

Reference to the appearance of tumor islet cells, observed fresh after *supra-vitam* staining with neutral red, makes it apparent that at least two types of cells occurred in the tumors: The majority had granules which did not color with neutral red; the granules of the minority did. Sometimes, particularly in tumor V, colored and uncolored granules were observed in the cytoplasm of a single cell. Differences in the colors assumed by the specific granules of a majority and minority of cells were detected in fixed and stained preparations. It is well to state immediately that we do not presume the types of cells which can be demonstrated supravitally by neutral red to be identical with those apparent in fixed and stained preparations. It is true that in each procedure the numerical ratio of one type to the other may be similar (tumor III*b*) and also that each division is based on the staining characteristics of the specific cytoplasmic granules. But for obvious reasons, it is not possible to apply the supravital and fixed tissue methods to the study of a single group of cells. To avoid confusion, therefore, we speak hereafter of the majority cells of fixed and stained preparations as type I cells, the minority cells as type II.

In a preparation from tumor III*b* fixed in the formaldehyde chrome sublimate and stained with Bowie's dye, the distinction between type I and II cells was most evident. The specific cytoplasmic granules of type I cells colored faint blue; those of the type II cells stained an intense blue. There was a similar difference between the two types when Bensley's neutral gentian was used. Following the use of Mallory azan stain, the specific cytoplasmic granules of type I cells colored lilac to pink; those of the type II cells, purple. A third variety of cell was

observed in preparations fixed in the formaldehyde chrome sublimate and stained with Mallory azan stain. The specific cytoplasmic granules colored red. Owing to the infrequent occurrence of these cells in tumor III*b*, and their nonoccurrence in the other tumors, they deserve but passing mention.

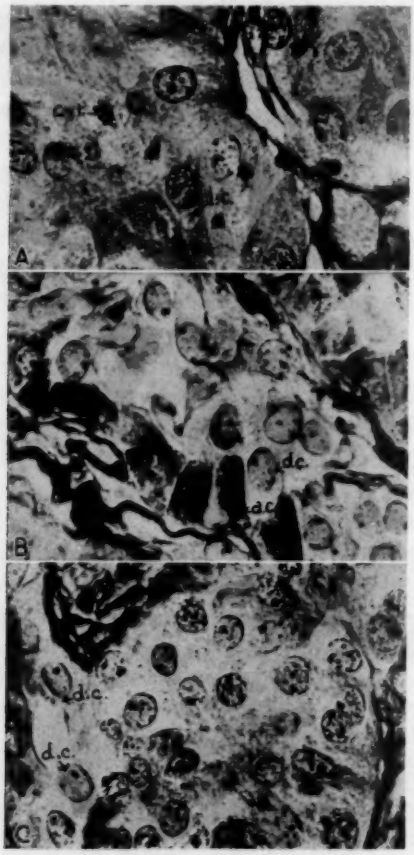

Fig. 2.—*A*, characteristic cell group from tumor III*b*. Note the intimate relationship of cells to the capillary channels, cytoplasmic inclusions (*cyt.*) and intracellular canals (*i.c.*). Formaldehyde chrome sublimate; Mallory azan. *B*, acinar cells becoming differentiated from duct epithelium in area of regenerating pancreatic tissue at the edge of tumor III*b* adjoining the pancreas. Acinar cells are indicated by *a.c.*; duct elements, by *d.c.* *C*, intimate relationship of duct and islet tissue in the vicinity of the central core, tumor III*b*. Duct cells are indicated by *d.c.* All photomicrographs are enlarged to 1,220 diameters.

In tumor III*b*, type II cells were fairly numerous and occurred singly or in clusters of from two to eight. In tumors I and II, they

were found in similar proportions. In tumor IV, type I cells were infrequent, and their specific granules somewhat larger than in tumors I, II and III*b*. In tumor V, the type II cells were not observed.

Can the type I and II cells of the adenomas be said to correspond to the major cell types of normal islets? Study of the material at our disposal does not warrant the conclusion that they can. We prefer to accept R. R. Bensley's decision that the type I cell is a B cell with modified staining reactions; the type II cell, a B cell corresponding to those of normal islets.

In preparations stained with hematoxylin and eosin the granules of the parenchymatous cells of the enterochromaffin and islet cell tumors are both eosinophilic and of approximately the same size. Besides, the cell form and appearance of the nucleus may present striking similarities. This led us to carry out the Masson test for enterochromaffin granules on tumors II, III*b*, IV and V. In each case, a piece of intestine known to contain enterochromaffin cells was fixed in Bouin's fluid and carried through the procedure with the tumor slide. In every instance the Masson reaction was positive for the granules of the enterochromaffin cells of the intestine and negative for the specific granules of islet cells.

Cytoplasmic inclusions of variable shape, but giving similar staining reactions, have been found in each of the tumors studied (fig. 2 *A*). Their occurrence in islet cell adenomas was detected by R. R. Bensley (Womack and his co-workers [3]). The inclusions were of most frequent occurrence in tumor III*b*, less often found in tumors I, II and IV and rare in tumor V. With Mallory azan stain, they colored bright red; with Bowie's stain, blue and with Bensley's stain, purple. They might be plastered on one surface of the nucleus ("sickle thickening," Bensley) or appear as globules or masses in the cytoplasm. In tumor III*b*, observed in mitotically active cells, they colored the same as the chromatin. These have been found only in tumor cells of type I, but never in type II, normal islet or acinar cells.

Mitotic figures were most frequently observed in type I cells of tumor III*b*. They usually occurred in cells with a reduced content of specific granules. In tumor V, only occasional mitotic figures were observed; in tumors I, II and IV, none.

In normal islets, cellular degeneration is of infrequent occurrence. Occasional cells with pyknotic nuclei and basophilic cytoplasm are observed, but there is no obvious equilibrium between cellular degeneration and replacement. There was little evidence of degeneration in tumor III*b*. Cells with pyknotic nuclei were found in small numbers, and occasional cell clumps were noted in which the nuclei were filled with droplets of nucleolar size. In tumors I, II, IV and V, cellular degeneration was frequent as evidenced by pyknosis, diffuse basophilia

of the cytoplasm and (tumor V) clumping of the mitochondria into droplets. Tumors I and II each had areas in which cell groups had been converted into débris. The cytoplasmic inclusions described have not been found in degenerating cells.

In all of the five tumors described, the majority of cells were sufficiently well differentiated to possess the characteristics of mature cells. In V, tumor cells in blood vessels, variability in the size of the cells and the many bizarre nuclear forms rich in chromatin were indicative of malignancy. However, this was probably of low grade, and by many the tumor undoubtedly would be classified as benign.

5. *Duct Components of Islet Cell Adenomas.*—Ducts occurred in all of the adenomas, which were similar in cell characteristics and arrangement to the small and medium-sized pancreatic ducts. They were least frequently met with in tumor V, but its large size did not permit a complete survey. The ducts occurred in the central cores of tumors III*b* and IV, in the connective tissue trabeculae of these and in tumors I and II.

Inferential evidence of the differentiation of duct into islet tissue was found in tumors I to IV (fig. 2 *C*). Notable areas were those about the central cores (III*b* and IV) and immediately adjoining normal pancreas. In these, hollow cords of islet cells frequently contained duct elements; single islet cells were found interspersed among the cells of ducts, and granules similar in size and staining characteristics to those of islet cells sometimes spotted the cytoplasm of an obvious duct cell. Similar sporadic occurrences were noted in the vicinity of the connective tissue trabeculae.

In the areas adjoining the pancreas in tumors III*b* and IV, evidence of the differentiation of duct into acinar tissue was also obtained (fig. 2 *B*). Sometimes ducts terminated in incomplete acini; again, cells containing a few zymogen granules and basophilic material (prozymogen?) were noted in the walls of ducts. Simple lobules, possibly in the process of building, were also seen. Differentiation of duct into acinar tissue did not occur elsewhere in the tumors.

6. *Islets of the Pancreas in Cases of Hypoglycemia.*—It might be expected that an overproduction of the hypoglycemic hormone would alter the appearance of the pancreatic islets. In all of the cases of tumor, portions of the pancreas adjacent to the tumors were available for comparison with the normal by the same variety of fixing and staining methods used in the study of the tumors. This survey included comparison with the normal, the following criteria being used: the proportion of alpha, beta and delta cells, richness of cells in specific cytoplasmic granules and evidence of degeneration. Apparent differences in the number of islets was not considered a good criterion (Bensley [7]). No

alterations could be demonstrated without the range of expected variability. A segment of pancreas removed from a 1 year old child (Dr. E. Graham) for chronic hypoglycemia also had normal appearing islets.

LITERATURE

Islet cell adenomas are not of rare occurrence. In 1926, Warren [20] collected fifteen cases from the literature and reported four others. All were discovered at necropsy. Since the hypoglycemic syndrome was not widely understood before that time, it is not surprising that the early tumors reported were not correlated with positive or negative evidence of hypoglycemia in the patients' histories.

Seale Harris [1] first suggested that the blood sugar level might be lowered and hypoglycemic symptoms become apparent owing to abnormal activity of the islet tissue of the pancreas. Since that time islet cell tumors have been discovered at the necropsy of patients who had had hypoglycemic symptoms. These cases have been reported by Thalhimer and Murphy,[21] McClenahan and Norris,[22] Smith and Seibel,[23] Terbrüggen,[24] Barnard,[25] Rabinovitch and Barden,[26] Cairns and Tanner,[27] and Wolf, Hare and Riggs.[28]

In two of the five cases reported by Smith and Seibel,[23] there was no history suggestive of hypoglycemia; in fact, one of the patients, during an attack of acute lobar pneumonia which shortly preceded death, "behaved as a total diabetic." [29] The parenchyma of the tumor had an arrangement similar to that of islet tissue; but the apppearance of the cells suggested a closer relation to duct than to islet tissue. The specific cytoplasmic granules characteristic of islet cells could not be demonstrated although the fixation was adequate (acetic and formaldehyde chrome sublimates). The other patient died of a primary liver cell carcinoma. In many cells of the tumor specific cytoplasmic granules, which stained as do the B granules of normal islets, were demonstrable.

The complete case reported by Wilder, Allen, Power and Robertson [30] first suggested operative intervention in spontaneous hypoglycemia

20. Warren, Shields: Am. J. Path. **2**:336, 1926.
21. Thalhimer, W., and Murphy, F. D.: J. A. M. A. **91**:89, 1928.
22. McClenahan, W. V., and Norris, G. W.: Am. J. M. Sc. **177**:93, 1929.
23. Smith, M. G., and Seibel, M. G.: Am. J. Path. **7**:723, 1931.
24. Terbrüggen, A.: Beitr. z. path. Anat. u. z. allg. Path. **88**:37, 1931.
25. Barnard, W. G.: J. Path. & Bact. **35**:929, 1932.
26. Rabinovitch, J., and Barden, F. W.: Am. J. M. Sc. **184**:494, 1932.
27. Cairns, R. M., and Tanner, S. E.: Brit. M. J. **1**:8, 1932.
28. Wolf, A.; Hare, C. C., and Riggs, H. W.: Bull. Neurol. Inst. New York **3**:232, 1933.
29. Death resulted from a pulmonary embolus which originated in the femoral vein.
30. Wilder, R. M.; Allen, F. N.; Power, M. H., and Robertson, H. E.: J. A. M. A. **89**:348, 1927.

due to suspected islet cell tumor. The first successful operation was reported by Howland, Campbell, Maltby and Robinson.[31] Since that time there have been five operative removals of adenomas of the islets of Langerhans at Barnes Hospital, St. Louis. Dr. A. O. Fisher removed two such tumors (one reported by Carr, Parker, Grove, Fisher and Larimore;[2] one diagnosed by Dr. L. F. Aitken, not yet reported); Dr. Evarts Graham, three (Womack, Gnagi and Graham;[3] Graham and Womack,[4] one not yet reported). Dr. E. R. Schmidt at the University of Wisconsin has removed one (Bast, Schmidt and Sevringhaus[32]). In each of these cases there was a successful outcome with complete alleviation of symptoms.

Architecturally and from the point of view of cellular differentiation, the pancreatic tumors giving rise to hypoglycemic symptoms have all resembled the islets of Langerhans. However, it may not be inferred that all tumors with well differentiated islet cells provoke hypoglycemic symptoms. One of the two cases without symptoms reported by Smith and Seibel illustrates this point. Doubtless other such tumors will be discovered at necropsy. The factors which may condition the severity of symptoms are enumerated on page 305.

COMMENT

The genesis of the islet cell adenoma is of particular interest in view of the researches of Bensley[33] and Grauer[34] on the origin of islet and acinar epithelium from the pancreatic ducts in young adult animals. Bensley has shown in rabbits that following ligation of the duct of Wirsung the acini and islets degenerate. Coincident with and following this degeneration, there is a restoration of islet tissue through the building of new islets from the ducts. Grauer demonstrated the regeneration of acinar epithelium from the ducts by similarly ligating the main duct, waiting until the acinar epithelium was completely destroyed, as proved by histologic control, and then reestablishing the duct connection with the intestine. With the free drainage of the duct system, the phenomenon of regeneration of acini from the epithelium of the duct was observed. In several cases the pancreas of young adult rabbits was completely regenerated. The epithelium of the duct is thus totipotent, capable of forming either acinar or islet tissue.

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31. Howland, G.; Campbell, W. R.; Maltby, E. J., and Robinson, W. L.: J. A. M. A. **93**:674, 1929.

32. Bast, T. H.; Schmidt, E. R., and Sevringhaus, E. L.: Acta chir. Scandinav. **71**:82, 1932.

33. Bensley, R. R.: Harvey Lectures, Philadelphia, J. B. Lippincott Company, 1915, vol. 10, p. 250.

34. Grauer, T. P.: Am. J. Anat. **38**:233, 1926.

In the central cores of tumors III*b* and IV of our series, ducts were plentiful. Centrally located ducts were also observed in the tumor reported by Howland and his co-workers.[31] Despite the occurrence of ducts in the trabeculae of tumor III*b* and crossing into it from the normal acinar tissue adjoining, no evidence was obtained to indicate that the central mass of ducts was connected with the ducts of the pancreas, though it is to be presumed that all ducts found in the islet tumors are of this origin. This question can be settled only by the study of serial sections through a small adenoma.

Around the central cores of tumors III*b* and IV, in the projections of the ducts into the parenchyma and also in the central cores themselves, inferential evidence of the differentiation of ducts into islet tissue was found (p. 304). It seems likely, therefore, that the ducts of the central core in these tumors served as one focal point for the differentiation of islet tumor tissue and may indeed have been the ultimate origin of a major portion of it. That differentiation from duct tissue alone does not explain the increase in size of the tumors is proved by the numerous mitoses observed in the islet cells of tumor III*b*.

At the outskirts of tumors II, III*b* and IV, in contiguity with normal pancreas, ducts were also present. In tumor III*b*, the capsule was broken in this area by the passage of ducts from normal pancreas into the tumor. In all those mentioned, differentiation of duct into both islet tumor and apparently normal acinar tissue was observed. However, evidence that a single duct might differentiate into both acinar and islet tissue could not be obtained. This, and the failure to find forming acini elsewhere in the tumors, might be taken to indicate that the epithelium of the duct, while totipotent, may be restricted in its ability to form either islet or acinar tissue by environmental or other (intracellular) influences.

Evidence concerning the likeness of tumor cells to A and B cells of normal islets must be examined with care. This question cannot be settled until the results from a much larger series are weighed. A survey of the present material suggests definitely that the type I cells of the adenomas have tumor characteristics; but in order that the staining characteristics of the tumor as compared with normal islet cells can be finally evaluated, future material must be fixed in 70 per cent alcohol and Hermann's fluid in addition to the fixatives previously prescribed. Bowie's and Bensley's stains should be applied to material fixed in these fluids and in aqueous chrome sublimate and the results compared with those of Lane.[11]

Our uncertainty regarding which cells of the tumors have granules that color with neutral red has already been alluded to. In tumor V, the study of fixed material would lead one to believe that all cells belonged to the same category (type I). Yet, in the fresh study, neutral red

stained the specific granules of a larger number of cells than in any of the other tumors, and cells were met not infrequently which had both colored and uncolored granules.

Tumor III*b* had numerous type II cells, yet fewer fresh cells proportionately stained with neutral red than in tumor V. It is at least evident, as previously intimated, that the type II and I cells of fixed material do not correspond, respectively, to fresh cells which do and do not stain with neutral red.

The stages of hyalinization as observed in the islets of the pancreas ("the most typical pancreatic lesion in diabetes mellitus," Warren [35]) and in the islet cell adenomas offer striking parallels. In both, the hyalin appears first about the vessels and pushes the cells progressively farther away from the vascular channels. Two possible origins of the hyalin of the islets have been discussed. Those favoring an epithelial origin point to the occurrence in the islets of cells the cytoplasm of which colors blue with Mallory stain. In a later stage, intracellular hyaline droplets are said to appear, these fusing to form hyaline masses about the capillaries. Warren pointed out, however, that the cells with blue-stained cytoplasm after Mallory's connective tissue stain appear in normal islets; and Bloom,[12] using the Mallory azan method, has found them so constantly that he has designated them as a third cell type, the D cell. Warren further stated that "the serial section method shows that the masses of hyaline are always in contact with the walls of the vessels of the islands, and always intercellular." He concluded: "Hyalinization of the islands of Langerhans . . . may be considered as due to production of intercellular substance by fibroblasts and possibly by endothelial cells." The evidence afforded by study of hyalinization in islet cell adenomas supports Warren's conclusions. Furthermore, the D cells with blue-staining cytoplasmic granules have not been found in the adenomas of our series.

Certain inferences may be drawn between the histologic appearance of the islet cell adenomas of our series and the amount of hypoglycemic hormone each was capable of producing, as judged by the severity of preoperative symptoms and (in two cases) by the insulin assay of a portion of the tumor. The patient having tumor III*b* showed the most severe hypoglycemic symptoms of the series. While the tumor was not large, it was actively growing; the majority of cells deviated to the least degree from the beta cells of normal islets; the cells immediately adjoined capillary channels. Next in order of severity of symptoms was the patient having the 500 Gm. tumor V. The ratio of weight between III*b* and V was in the order of 1:250. The appearance of the majority of

35. Warren, Shields: The Pathology of Diabetes Mellitus, Philadelphia, Lea & Febiger, 1930.

cells deviated more markedly from that of the beta cells of normal islets than in any other tumor of the series; the cells were most frequently separated from the vascular channels by accumulations of hyalin. The insulin assay was relatively low. The patients having tumors I and II grade next in order of severity of symptoms. These tumors, like III*b* were small and had areas in which the cells were widely separated from the vascular channels by hyaline masses; areas of degeneration were apparent in each. However, the majority of healthy appearing cells deviated little from the beta type; and tumor II had an area in which the healthy appearing cells closely adjoined the capillaries as in III*b*. The patient having tumor IV had the least severe symptoms of hypoglycemia. The tumor was the smallest of the group; it contained a considerable area of degeneration, and a majority of the cells deviated markedly in appearance from normal beta cells, though not as much so as in tumor V. Summarizing, then, the apparent factors which determine the severity of symptoms are: (1) degree of similitude of the majority of cells to the beta cells of normal islets; (2) the access of cells to the vascular channels; (3) size of the tumor, and (4) evidence of degeneration. Another factor which must be considered is the functioning of the normal islets of the pancreas.

The evidence developed by Bensley and confirmed by us indicates that these adenomas may have an age cycle. Do some of them degenerate into nonspecific masses after a period of active existence? Tumor III*a*, a nonspecific mass with a calcified core, removed from the pancreas that lodged III*b*, was very similar in histologic appearance to a nodule of reddish friable tissue which occurred in the capsule of tumor III*b* (p. 298). The possibility is evident that the nonspecific mass, III*a*, may have been an active tumor which produced hypoglycemic symptoms in the patient before the development of the apparently young tumor III*b* (Graham and Womack [4]). Removal of this mass gave little temporary relief, and after three days did not result in even a partial amelioration of symptoms.

Changes must be sought for in the pancreatic islets of patients having islet cell adenomas producing hypoglycemic symptoms. Such alterations would be useful if they proved specific. A biopsy on the pancreas in a suspected case without visible or palpable evidence of a tumor at the exploratory operation might then prove an excellent diagnostic procedure. Any alterations found must be analyzed with reference to the patient's carbohydrate tolerance after operative removal of the adenoma, since adenomas have been found in the pancreases of diabetic patients (Büchner [36]).

36. Büchner, F.: Klin. Wchnschr. **11**:1494, 1932.

SUMMARY

Five tumors of the pancreas, operatively removed, have been identified as islet cell adenomas, verifying in each case the preoperative diagnosis of hypoglycemia due to suspected tumor. Although the adenomas varied in size, gross appearance and apparent age, the majority cell type of each was closely allied to the beta cells of the normal islets of Langerhans, but possessed definite tumor characteristics. In only one of the five tumors was there evidence of malignancy.

The staining reactions of the specific cytoplasmic granules in the majority of tumor cells of each deviated sufficiently from those of the beta cells of normal human islets to lend support to the hypothesis of dysinsulinism.

In none of the cases did the histologic picture of the islets of Langerhans of the pancreas containing the tumor indicate the suppression of function that might be expected to parallel the prolonged secretion of excessive amounts of the hypoglycemic hormone.

MALFORMATIONS OF THE HEART OF THE NEW-BORN

CONGENITAL LESIONS SUGGESTIVE OF AN INFLAMMATORY ORIGIN

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Etiologically, congenital lesions of the heart can be explained either on a developmental or on an inflammatory basis. Formerly most abnormalities were considered the result of inflammatory processes, and lesions, cicatrized or active, in the region of the malformation were assumed to be of inflammatory origin. It was not until Rokitansky, introducing the term "fetal endocarditis," laid down certain rigid microscopic criteria that the rarity of lesions with an inflammatory genesis was appreciated. They occur more frequently in the left side of the heart. In 1907, Mönckeberg [1] could collect from the literature but 12 reports of such cases, to which he added a case of his own. Since then, Fischer,[2] Nagel,[3] Loeser,[4] Kockel,[5] Ganeff [6] and Le Tulle [7] have described similar cases on an inflammatory basis with marked stenosis of the aortic opening.

Keith, on the contrary, on the basis of a study of 270 cases of congenital defects of the heart, reached the conclusion, because of lack of scars and because of the concomitant anomalies in other parts of the body, that changes are the result, not of infection, but of a disturbance of the conditions under which the fetus develops. Vierordt [8] estimated that other abnormalities, such as harelip, syndactylism, horseshoe kidney and hypospadia, are found in about 10 per cent of the cases of congenital heart disease.

From the Clinic of the Woman's Hospital.

1. Mönckeberg, J. G.: Verhandl. d. deutsch. path. Gesellsch. **11**:224, 1908.
2. Fischer, B.: Frankfurt. Ztschr. f. Path. **7**:83, 1911.
3. Nagel, W.: Ein Beitrag zur Kasuistik ueber angeborene Herzfehler, Dissert., Freiburg, 1908.
4. Loeser, A.: Virchows Arch. f. path. Anat. **219**:309, 1915.
5. Kockel, R.: Verhandl. d. Gesellsch. deutsch. Naturf. u. Aerzte, Koeln **80**:39, 1908.
6. Ganeff, M.: Ueber angeborene Stenose und Atresie der Aorta durch foetale Endokarditis, Dissert., Würzburg, 1910.
7. Le Tulle: Presse méd. **22**:432, 1914.
8. Vierordt: Angeborene Herzkrankheiten, in Nothnagel: Specielle Pathologie und Therapie, Vienna, A. Hölder, 1898.

Rauchfuss [9] emphasized that septal defects constitute the main indication of a developmental anomaly. The absence of septal defects, however, does not always predicate an inflammatory origin, as is shown by descriptions of cases of cor pseudotriloculare without septal defects, in which the developmental origin cannot be doubted.

Septal defects occur much less commonly in association with aortic stenosis and atresia than with the corresponding lesions of the pulmonary valve; according to Vierordt, there are about three times as many cases of aortic stenosis and atresia without, as with, septal defects. Theremin [10] collected 17 such cases, in only 2 of which there was a defect of the septum, and in Abbott's [11] series of 6 cases there was no septal defect in 5. It is obvious that in cases without septal defect, the determination of whether the genesis was developmental or inflammatory is much more difficult. In the cases in which the septum is open, it must be recalled that the aortic valve is completely formed in the seventh week of intra-uterine life, and the intraventricular septum by the end of the eighth week; hence, in order to explain the nonclosure of the septum, it must be assumed that the process of stenosis, if due to inflammation, commenced just at the end of the seventh week, and ceased before the end of the eighth week, when the septum normally closes. This, as Vaquez [12] stated, is inadmissible.

Another factor which is contradictory to the theory of an inflammatory origin is that congenital lesions of the heart may be hereditary and familial. De la Camp found almost identical cardiac malformations in 2 sisters and 4 brothers. Burwinkel described the case of a man of 54, who had been cyanotic since birth, as were his mother and grandmother and 2 of his 3 children.

According to Rauchfuss,[9] the time of development of a lesion must be placed before the twentieth week of life if a recessus formation of the right ventricle (a funnel-shaped extension of this cavity, pointing to the left and surrounding the left ventricle) is encountered.

In general, the size of the right ventricle in relation to that of the left is an indicator of the time of occurrence of the lesion; the earlier a disturbance takes place, the larger will be the right ventricle, which, with a few exceptions, hypertrophies in response to functional demand and carries the burden of the whole circulation.

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9. Rauchfuss, C.: Die angeborenen Entwicklungsfehler des Herzens und die Foetalkrankheiten des Herzens und der grossen Gefaesse, in Gerhardt: Handbuch der Kinderkrankheiten, Tübingen, H. Laupp, 1878, vol. 4, p. 1.

10. Theremin, E.: Etude sur les affections congénitales du coeur, Paris, Asselin & Houseau, 1895.

11. Abbott, M. E.: Congenital Heart Diseases, in Osler, William, and McCrae, Thomas: Modern Medicine, ed. 3, Philadelphia, Lea & Febiger, 1927, vol. 4.

12. Vaquez, H.: Diseases of the Heart, Philadelphia, W. B. Saunders Company, 1924.

Inflammation is possible as soon as vessels appear in a system, and although vascularization occurs early in the embryo, it is Fischer's [2] opinion that the rare occurrence of inflammation in early intra-uterine life is due not to failure of development of the vessels but to a special immunity of the embryonal system against infection. In the early period the organism lacks cell receptors for toxins; as intra-uterine life progresses, the receptors develop, and as gestation reaches its end, the embryo is much more susceptible to infections. Ribbert [13] emphasized the remarkable fact that in congenital syphilis, in which there is often an enormous growth of spirochetes in the fetal body, infection of the valves of the heart does not take place. Syphilitic changes in the valves have never been found in the new-born (B. Fischer and Herzheimer). In general, therefore, it appears that the fetal valves are seldom the site of infectious lesions. In the early years of extra-uterine life also this specialized immunity seems to hold good. In 1,000 autopsies on children under 3 years of age, Ribbert found not a single case of endocarditis. Other observers, however, have reported a few cases occurring in this age group. In the second decade of life, as is well known, the number of endocardial infections increases enormously.

The present view thus tends to assign the majority, if not all, of the congenital defects primarily to abnormalities of development. However, in the following report of 2 cases of morphologically similar abnormal hearts in new-born infants, the results of detailed histologic examination tend to prove the inflammatory nature of the lesions.

REPORT OF CASES

CASE 1.—*History.*—A boy was born, by low forceps delivery, of a healthy primipara, 28 years old. The prenatal period was uncomplicated. The baby lived forty-eight hours, during which he showed severe progressive cyanosis and respiratory embarrassment.

Gross Postmortem Examination.—There were no external abnormalities. The internal organs were in normal position, and there were no abnormalities except in the heart, which occupied the largest part of the thoracic cavity. The pericardium was glistening and contained about 10 cc. of clear fluid. The anterior aspect of the heart and the apex were formed by the right ventricle; the left ventricle was small and appeared as an appendix to the right. The incisura cordis was more than 1 cm. deep and was situated to the left and posteriorly. Both auricles appeared large. The longitudinal sulcus was situated near the left margin and was only 1.5 cm. long. The posterior aspect of the heart was supplied mainly by the right ventricle, which measured at its widest 3.3 by 3.2 cm., while the left measured only 2.2 by 1 cm.

The large vessels originated normally. The pulmonary artery, which originated without a distinct conus, was 2.5 cm. wide when opened. The branches to both lungs were given off in the normal way. The ductus Botalli was 1.3 cm. wide. The

13. Ribbert, H., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol. 2.

descending aorta appeared as the continuation of the pulmonary artery. Below the origin of the right carotid artery the aorta was 1.5 cm. in diameter; the aortic ostium, however, was narrow, being less than 0.5 cm. in diameter. An attempt to enter the aorta from the ventricle failed, as the probe was arrested by an elastic resistance at the level of the aortic valve. This obstruction was due to the coalescent leaflets of the aortic valve which formed a folded membrane on the inferior surface of which were the fused noduli arantii. A narrow slit, 2 cm. long, in the center of the membrane was the only communication between the aorta and the left ventricle. In addition, the lateral portion of the membrane, at its upper margin, was intimately adherent to the aortic wall. The sinuses of

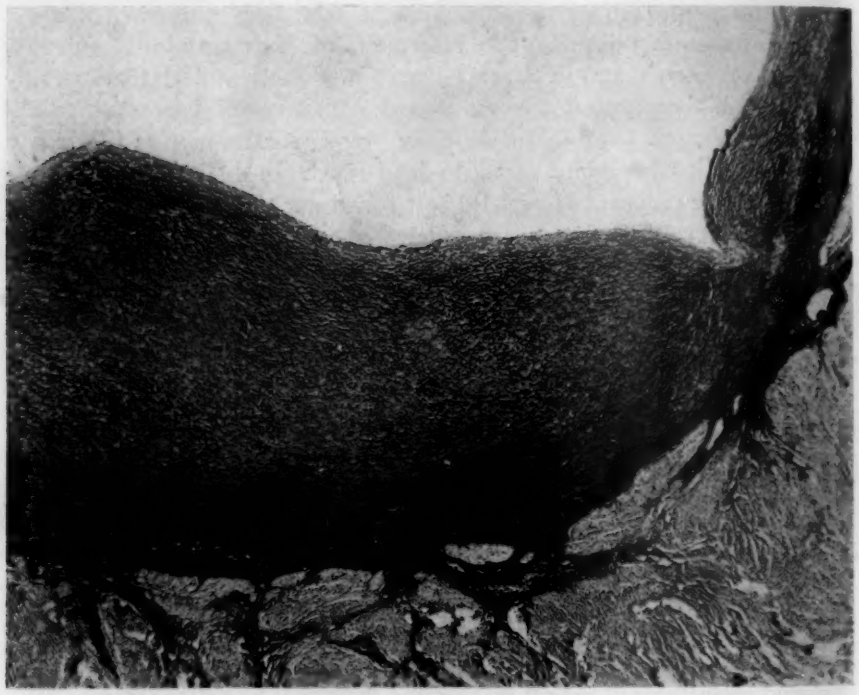

Fig. 1 (case 1).—Thickened endocardium of the left ventricle (elastica stain).

Valsalva appeared high and flat. The commissures were unusually long. The coronary arteries originated normally; their orifices were rather wide.

The right ventricle appeared normal from inside; the medial papillary muscle, however, was only 6 mm. long, while the others measured from 1.2 to 1 cm. The thickness of the wall, without trabeculae, was 3 mm.

The left ventricle as a whole was turned backward, almost forming a recessus to the right ventricle. The endocardium here was transformed into a pale grayish, smooth layer of an average thickness of 1.5 mm. The anterior papillary muscle was partly coalescent with the endocardial thickening, only a short upper portion extending from the wall. The mitral valve was represented by the posterior cusp only, as the aortic cusp was lost within the thickening of the wall, causing shortening of the corresponding papillary muscle. The chordae tendineae

were short anteriorly and long posteriorly, and the spaces between them were partly filled by a reddish, gelatinous tissue. The intima of the left auricle was normal. The foramen ovale was patent, and its diameter was about 5 mm.

The epicardium did not show anything of note.

Histologic Postmortem Examination.—Left Ventricle: The thickened endocardial layer (fig. 1) consisted of elastic connective tissue and in places attained a thickness equal to a third of the myocardium. The borderline between the endocardium and the myocardium generally was well marked; in many places, however, the elastic layer projected into the muscle with long extensions that continued along the course of the blood vessels. The elastic fibers were arranged

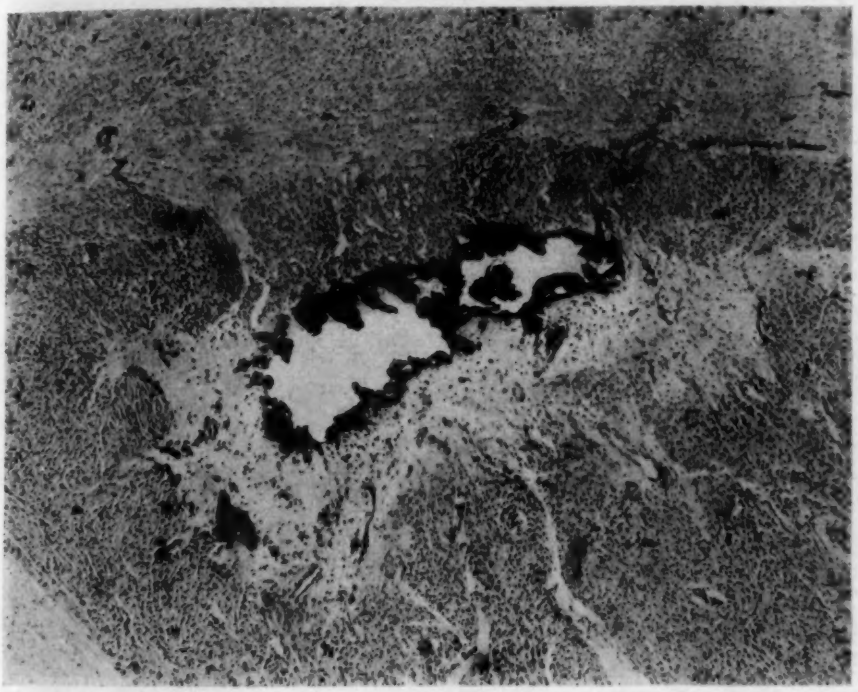

Fig. 2 (case 1).—Calcification in the myocardium of the left ventricle.

parallel to the inner myocardial surface and were coarser and denser in the basal layer in the parts projecting into the myocardium. Congested capillaries were found in the endocardial thickening close to the myocardium. Near the papillary muscle there was a large necrotic area with extensive central calcification. Multiple calcified deposits were found in interstitial spaces occupied by hyalinized connective tissue (fig. 2). In the adjacent muscle tissue the capillaries were increased in number and engorged.

The fibers of the heart muscle showed in places irregular arrangement, atrophy and vacuolation. The nuclei varied considerably in size and shape, a condition which might be attributed to the embryonic stage of the muscle. Aggregations of small round cells were frequently seen (fig. 3), and numerous small necrotic areas and islands of cicatricial connective tissue were found in the muscle sub-

stance; there were considerable thickening and hyalinization of the blood vessel sheaths and extravasation of blood.

Right Ventricle: There were numerous localized necroses and some round cell infiltration in all layers of the heart muscle; there was no increase of the elastic tissue. The muscle cells varied in size and structure and were somewhat irregularly distributed. The sheaths of the walls of the blood vessels were thickened in many instances.

Left Auricle: Endocardial thickening of the same type as in the left ventricle, though of lesser extent, was found, likewise with projections into the myocardium. The muscle exhibited marked irregularity in structure.

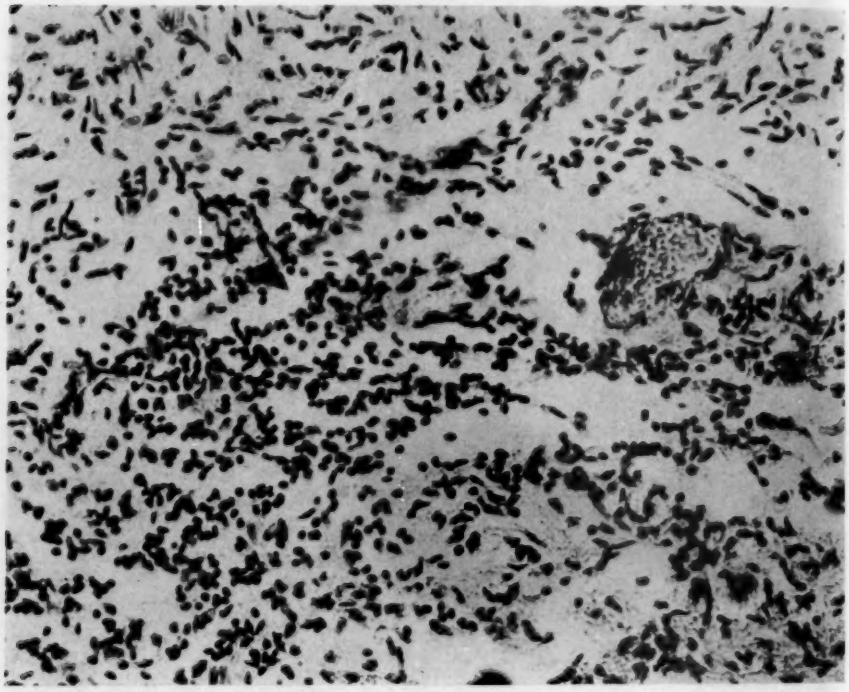

Fig. 3 (case 1).—Small round cell infiltration in the myocardium of the left ventricle.

Right Auricle: The endocardium was normal; the myocardium showed irregularities of the muscle tissue.

Septum: There were no changes aside from structural irregularity of the muscle.

Papillary Muscle of the Left Ventricle: Necrosis occupied most of the center, with extensive calcification, as described in connection with the left ventricle. The adjacent muscle tissue was preserved only in fragments, and there were marked increase and engorgement of the capillaries. A broad peripheral layer composed of elastic connective tissue formed the outer area. The extent and shape of the calcification did not suggest an origin in a former blood vessel.

Mitral Valve: In cross-section (fig. 4) the valve represented an irregularly folded band and consisted of loose, regularly arranged connective tissue with scanty elastic fibers. The endothelium was well preserved in areas. The opposite wall of the ventricle showed the endocardial and muscular changes that have been described. There was no trace of an active or chronic inflammatory lesion.

Aortic Valve: The different parts of the valve showed variation in density without inflammatory changes. Elastic tissue could not be demonstrated within the valve proper, since the elastic surface layer of the wall of the ventricle ends at the origin of the valve.

The right atrioventricular valve, the pulmonary valve and the aortic wall did not show unusual appearances.

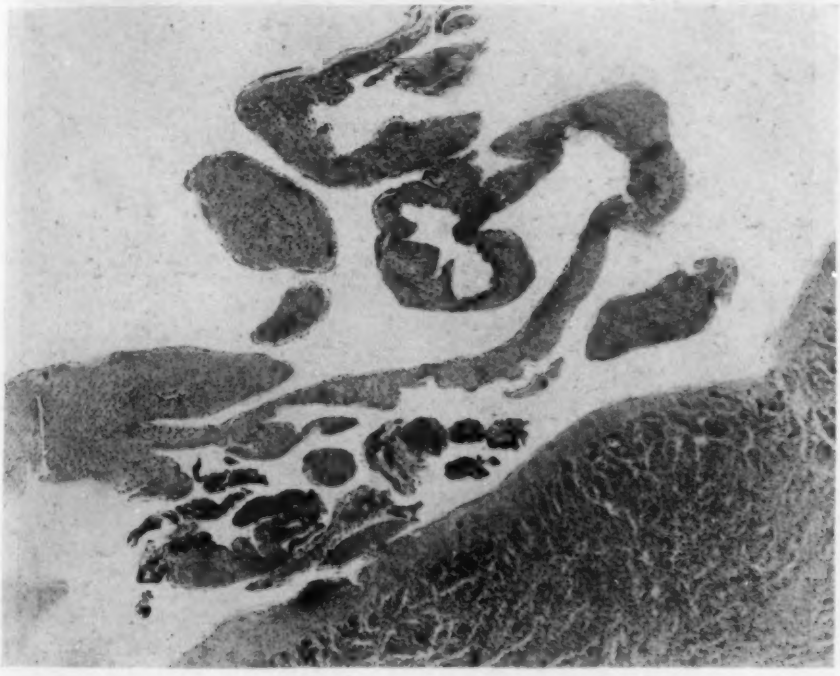

Fig. 4 (case 1).—Transverse section through the mitral valve near the free margin, showing fusing and clumping of cusps.

Case 2.—*History.*—A boy, born at full term, by spontaneous delivery, of a healthy secundipara, 28 years old, lived for nine days, during which time he presented the clinical symptoms of cyanosis of various intensity with attacks of apnea.

Gross Postmortem Examination.—The patient was a new-born boy, 54 cm. long, with all the external signs of maturity. There were no abnormalities except cyanosis and scattered petechial hemorrhages.

When the chest was opened the lungs were found to be pushed backward by the heart, which was much enlarged and occupied the left side and part of the right side of the thoracic cavity. The pericardial sac contained about 5 cc. of clear, serous fluid and showed a good sheen.

The heart presented an unusual appearance. The anterior aspect was almost entirely made up of the right ventricle and auricle, the latter being greatly distended and congested. The left ventricle was about a third of the size of the right and simulated a dome-shaped protrusion of the right ventricle. The incisura cordis was shifted to the left and was about 0.5 cm. deep. The apex consisted entirely of the right ventricle. The main measurements of the heart were: width at the base, 5 cm.; width of the right ventricle at the base, 3.7 cm.; longest distance from the top of the right auricle to the apex, 5.2 cm.; distance from the base of the left ventricle to the apex, 4.2 cm. The longitudinal sulcus was shallow and entirely effaced near the base; it measured 2 cm. in length. There was a

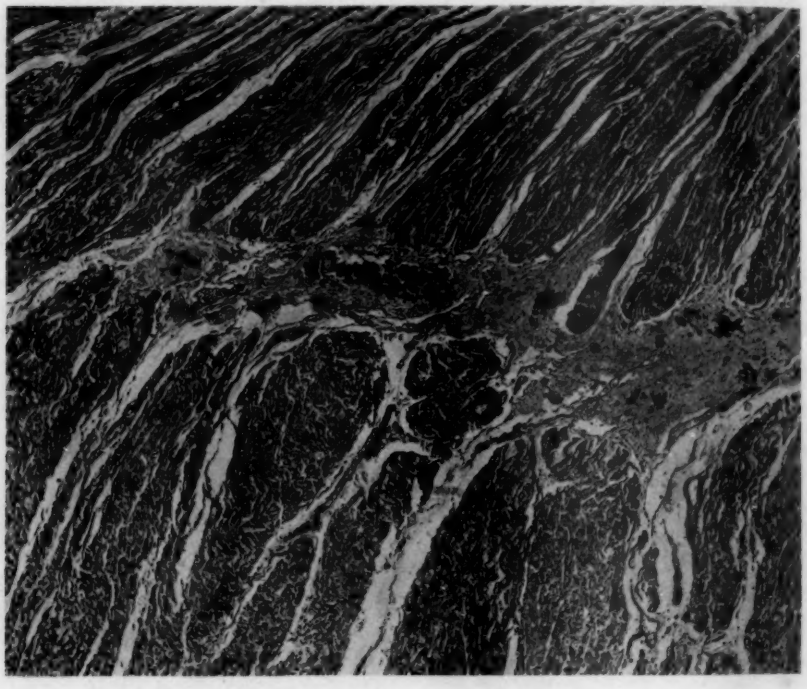

Fig. 5 (case 2).—Diffuse calcifications in the myocardium of the left ventricle.

subserous hemorrhage about 1 cm. in diameter at the base, near the origin of the longitudinal sulcus. Numerous pinpoint-sized subserous hemorrhages were scattered over the pulmonary conus and the ascending aorta. The configuration of the large vessels was unusual. The pulmonary artery in its ascending portion formed an aneurysmal dilatation, with a widest diameter of 9 mm., which after a course of 10 mm. gave off its two branches. The main stem, however, continued with a narrowed diameter of 5 mm. upward to the aorta, thus corresponding to a large ductus Botalli. It united with the aorta after the latter had given off the left subclavian artery. The aorta originated normally. In its ascending portion it formed a wide arch, which varied in width in its different parts but, on the whole, was considerably narrower than the pulmonary artery. It measured 6.5 mm. in diameter at its origin, 4 mm. between the origin of the innominate artery and that of the left carotid artery and dilated to a diameter of 5.5 mm. before

its junction with the wide portion of the pulmonary artery, referred to as the ductus Botalli. Owing to the inversion of the normal proportions in the dimensions of the two vessels, it seemed as if the aorta would empty into the pulmonary artery. From here on the aorta widened suddenly, attaining a diameter of 8 mm., and formed a second arch in its descending portion. The arch of the aorta and the pulmonary artery could easily be probed from the descending aorta through the ductus Botalli. The main vessels to the head and the arms originated in the normal way.

Examination of the chambers and valves revealed the following: The wall of the left ventricle was uniformly 9 mm. thick; the external layers were homogeneous, and the inner layers were of coarser texture, with intertwining, whitish fibers. The inner surface of the whole ventricle was invested with a dense, whitish layer, varying in thickness from 1 to 2 mm. The inner architecture of the ventricle was largely effaced; the papillary muscles were obliterated; the trabeculae carneae could be vaguely distinguished under the endocardial coat. The mitral valve presented only one free leaflet (the aortic); the short chordae tendineae fused directly with the endocardium; the second leaflet was completely merged with the endocardium and its contours could be distinguished only vaguely. The atrioventricular ostium was moderately stenosed. The left auricle was small and of normal shape, and the endocardium did not reveal an unusual appearance.

The wall of the right ventricle was 6 mm. thick, enlarging to double this width in the area of the conus. The cavity was greatly enlarged, forming a bulging pouch in its anterior upper portion, which corresponded to the distended conus. The tricuspid valve was normally developed. The right auricle did not present any unusual appearance except for moderate dilatation. The endocardial layer in both right chambers appeared normal. The aortic ostium was narrowed, and the aortic valve was composed of two triangular flaps attached at their bases to the ostium and projecting with free edges upward into the aortic lumen; the flaps were composed of gelatinous tissue with smooth surfaces, thrown into longitudinal folds. The lumen of the ostium was almost occluded by these structures, which left an opening only 1.5 mm. in diameter. The pulmonary ostium appeared normal.

The other organs did not reveal unusual changes.

Microscopic Postmortem Examination.—Left Ventricle: As in case 1, the endocardium was converted into a broad layer of noncellular elastic connective tissue, which in some areas was more than half the thickness of the muscle wall. The borderline toward the myocardium was irregular and frayed; the muscle receded, and the connective tissue projected into the myocardium with finger-like extensions frequently following the course of large blood vessels. This unusual endocardial thickening was continued in the left auricle, but ended gradually at some distance from the origin of the mitral valve. Large blood vessels in dense distribution, occupied or surrounded by calcified depositis, were found near the apex in the endocardial coat where it reached its largest extent. In an area nearby extensive necrosis with calcification was encountered in the muscle; in its environs vascularization and congestion were most extensive.

The subendocardial muscle layer was composed of atrophic fibers with wide interstitial spaces. There were marked irregularity of the muscle cells, particularly at the site of the origin of the mitral valve, and general hyperemia and engorgement of the capillaries, which were greatly increased in number in this part of the myocardium. In an area of the septum the subendocardial muscle layer was intersected by a strand of hyalinized connective tissue with numerous

scattered calcified deposits of varying extent, some of them apparently surrounded by remains of vessel walls (fig. 5). Round cell infiltration, necrosis or connective tissue scars were not found in this part of the myocardium. The muscle fibers became more regular in appearance and arrangement toward the periphery of the ventricular wall. The sheaths of the blood vessels in all parts showed extensive thickening and hyalinization. The most extensive pathologic changes in the myocardium were evident near the attachment of the papillary muscle of the left ventricle where subendocardially diffuse necroses and irregularities of muscle fibers and nuclei were apparent. The tissue in the distal portion of the muscle gradually became regular, and the attachment of the mitral valve was normal.

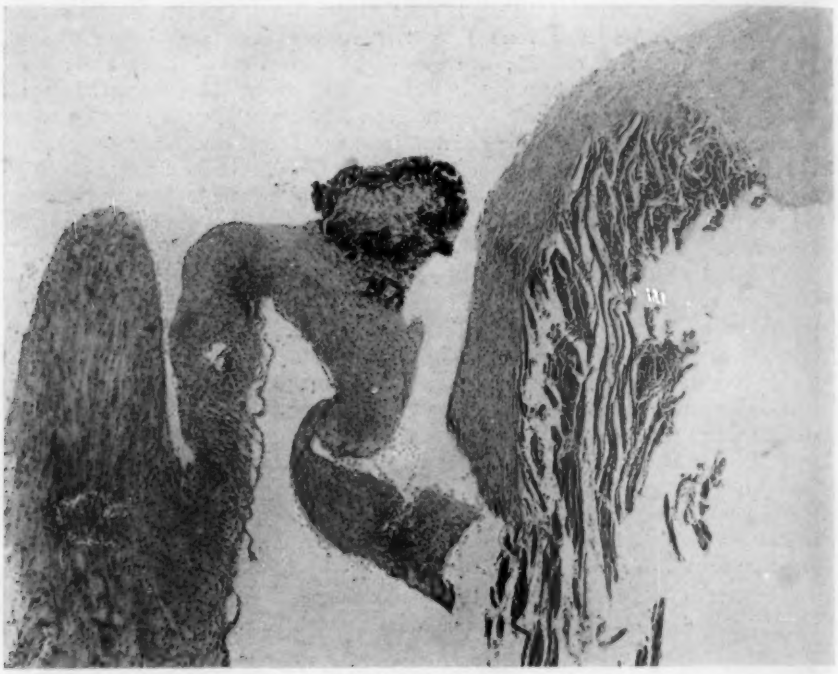

Fig. 6 (case 2).—Transverse section through the tricuspid valve near the free margin, showing calcification.

The mitral valve in longitudinal section presented an irregularly folded band composed of loose connective tissue with irregular cellular distribution. Some elastic fibers were found at the edges, and in some other areas there were clumps of opaque matter apparently corresponding to the gelatinous material seen grossly on the surface of the valve. The endocardium was well preserved in many places. There was no trace of inflammation as evidenced by round cell infiltration or vascularization, and there were no necroses or areas of condensed stroma indicative of a previous lesion.

The tricuspid valve was built of very loose connective tissue; the ground plate was little developed. On the upper edge of the atrial surface there was a large, knoblike body composed of loose connective tissue in the center and a

circular border of dense calcified deposits suggestive of derivation from a productive inflammatory process (fig. 6).

The aortic valve showed uneven contours, and several finger-shaped projections extended from it toward the aortic lumen. The ground plate was thicker than usual. The endocardium of the aortic surface was thickened and fused imperceptibly with the central plate of connective tissue; it contained large blood vessels which in a longitudinal section of the valve appeared as a chain of transverse sections. The endocardium of the ventricular surface did not show vascularization.

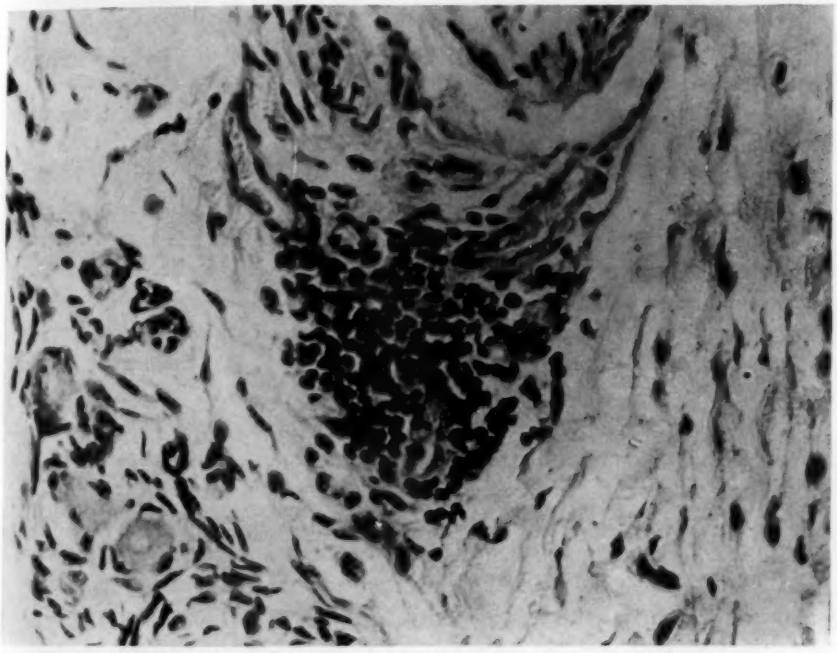

Fig. 7 (case 2).—Aggregations of round cells in the aortic adventitia.

The aorta and the pulmonary artery did not reveal structural alterations; however, dense aggregations of round cells were encountered in two places in the adventitial tissues of the aorta (fig. 7).

The lungs showed slight congestion and partial atelectasis. There was mild fatty degeneration of the liver. The kidneys showed congestion.

COMMENT

In evaluating the etiologic factors, macroscopic and microscopic changes are taken into consideration, but the histologic picture only is accepted as the final criterion for diagnosis.

In both cases the abnormalities were confined to the pathologic changes of the heart. This condition points to an etiologic factor acting

locally, since in the presence of developmental abnormalities in one organ multiple abnormalities in other organs commonly occur. A hereditary etiologic factor cannot be traced in either case since the parents and families of both infants were free from congenital abnormalities; the mother of the patient in the first case subsequently gave birth to two healthy babies. This further corroborates the theory of an inflammatory genesis, because in a high percentage of developmental lesions of the heart a familial predisposition can be demonstrated.

In both cases the malformation was evident chiefly in the left side of the heart; there was involvement of the proximal portion of the aorta, but the interventricular septum was completely developed, without defects. In the combined lesion of malformation and endocarditis which is represented by these two cases, involvement chiefly of the left ventricle corresponds, according to Rauchfuss, to the postnatal type of primary endocarditis, since in cases of developmental deformities of the heart with secondary endocarditis the inflammatory lesion is invariably seated in the right side of the heart.

The absence of a septal defect in both cases indicates that the development of the abnormalities must be assigned to a period later than the seventh or eighth week of intra-uterine life because the septum normally is closed by this time. Furthermore, at this early stage embryonal tissue is not considered to be susceptible to infection.

These morphologic changes, which are generally considered indicative of an inflammatory genesis, were abundantly substantiated by the histologic observations. In both cases there was an almost identical thickening of the endocardium, projecting into the myocardium, with extensive formation of elastic connective tissue. This picture corresponds to the phase of healing in a postnatal inflammatory condition and consequently indicates a reparative process in these cases. The extensive development of new capillaries and capillary leukocytosis are usual concomitants or sequelae of inflammation. In both cases the most extensive lesions were found within the papillary muscles (similar to Fischer's case), where necroses and calcifications indicate the past influence of toxic processes. That the papillary muscle is the seat of the most prominent lesion is apparently the result of increased functional demand. Round cell infiltration and capillary leukocytosis were found within the myocardium only in case 1; they may be considered as proof of an inflammatory genesis. The presence of focal necrosis and connective tissue scars furnishes additional support for this assumption.

That traces of past inflammation were least distinct in the valves proper can be explained by the fact that the valvular connective tissue possesses great regenerative power, particularly during fetal life. Consequently, in our cases the connective tissue of the valves was only

slightly inflamed or not diseased, while the endocardium on their surfaces showed extensive pathologic changes and the valves proper revealed severe deformities. Regarding the time of occurrence of the lesion, there was no apparent illness of the mother or any fault of environment which would give a clue to the transmission of infection. The onset of the disease obviously can be placed in an earlier period in case 1 than in case 2, since in the former instance the morphologic changes were much more extensive and there was obviously a greater disproportion between the right and the left ventricle, a condition which is based on a longer period of functional compensation and led in this case to a recessus formation of the left ventricle.

SUMMARY

Two cases of malformation of the heart and aortic stenosis in the new-born are described. In view of the anatomic and histologic observations the malformations may be considered secondary to a primary fetal inflammation.

COLLOID-CHEMICAL PROPERTIES OF SOME PROTEIN-AMINE COMPOUNDS

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When the water-holding capacity of living tissues [1] was first declared to be dependent on their content of hydrophilic colloids (chiefly proteins), it followed as a corollary that edema [2] is merely a state in which the normal degree of hydration is heightened. A cause of edema, then, was any agency which under the conditions operative in the living organism might so affect the (protein) tissue colloids that they acquired an increased capacity for swelling. First in this category was placed an abnormal accumulation or production of acids in the involved tissues,[3] and second, the accumulation of certain nitrogen compounds,[4] particular emphasis being laid on the amines, especially those which, biologically speaking, are poisonous.

This paper deals further with the second class of compounds. Their effects on a pure protein (casein) received this detailed study because of the importance which such an action is believed to have for the better understanding of what happens in the "inflammatory" types of edema. The acid factor alone yields a fairly complete explanation of the swelling that characterizes the noninflammatory edemas (such as those of heart disease, carbon monoxide poisoning and intoxication with various metals); and the therapeutic reduction of such edemas through the use of alkalis and of various neutral salts may be taken as proof of the correctness of the general concept. But in the inflammatory types (such as those encountered in conjunction with local or generalized infections) mere neutralization of acid with alkalis and salting leave a considerable fraction of the edema unaffected. The primary cause for this fraction of the edema must, therefore, be different, as must also

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1. Fischer, M. H.: Physiology of Alimentation, New York, John Wiley & Sons, Inc., 1907, pp. 187 and 267; Am. J. Physiol. **20**:330, 1907; Arch. f. d. ges. Physiol. **124**:69, 1908; **125**:99, 1908.

2. Fischer, M. H.: Physiology of Alimentation, New York, John Wiley & Sons, Inc., 1907, p. 268; J. A. M. A. **51**:830, 1908.

3. Fischer, M. H.: Œdema, New York, John Wiley & Sons, Inc., 1910, p. 99; footnote 2.

4. Fischer, M. H.: Science **38**:486, 1913; Kolloid-Ztschr. **14**:215, 1914.

be the type of the increased hydration observed. We have looked on the action of the various amines that are produced in infected areas (and produced also as the degradation products of sterilely injured protoplasm) as such a second cause for swelling and have seen the explanation of the therapeutic failure of alkalis and salts on this fraction of the edematous process in their inability to dehydrate the amine hydrations of protein. The next paragraphs present in summary further facts bearing on the general problem, discussing, in particular, the effects of various amines on the swelling, the liquefaction and the solution of a water-insoluble protein, casein.

EXPERIMENTS

In these experiments we employed a casein of stock origin, free from other proteins, carrying about 10 per cent of water and practically ashless.[5] After low temperature drying, it absorbed (in our terminology "dissolved" or "combined with") 2.12 times its own weight when thrown into water. The control tubes in the following experiments illustrate this initial swelling of the native, or neutral, protein. Its solubility *in* water (better expressed by saying its "miscibility" with water as judged by the production of an optically homogeneous mixture) was so low that the liquid above the swollen casein mass gave no reaction to the ordinary tests for protein. For purposes of further discussion it is necessary to keep these concepts of the solubility of the protein *for* water and that of its solubility *in* water carefully apart. The two properties are totally different,[6] and while in certain lyophilic colloid systems (particularly those of the liquid-liquid type as opposed to those of the solid-liquid type) the passage from one element to the other may be perfectly smooth (as when blood serum or any of the liquid soaps is mixed with water), solution of the protein in water is not a mere continuation of swelling. This fact will appear again in the experiments to be described.

For purposes of orientation the effects of adding increasing amounts of an acid to a standard weight of neutral casein in the presence of a given quantity of water are shown in *A* in the accompanying illustration. In this experiment, as in all subsequent ones, the mixtures were made in the cold, the photographs showing the appearance of the reaction mixtures at the end of twenty-four hours. Except for the control tube (*0*) which contained 12.5 Gm. (an aliquot portion) of protein with 100 cc. of water, the remaining tubes carried equal volumes of increasingly stronger solutions of hydrochloric acid (from hundredth-normal to tenth-normal). Progressively greater swelling may be noted in tubes *3* and *4;* in the remaining tubes the mixtures set to solid jellies which,

5. Harris' casein with 0.59 per cent ash.
6. Fischer, M. H.: Science **42**:223, 1915.

with an increase in the acid, became progressively more (optically) homogeneous. As commonly said, the casein in these tubes had "gone into solution," but that this was only partially true is proved by the fact that the resulting contents of the tubes were still as viscid as egg white.

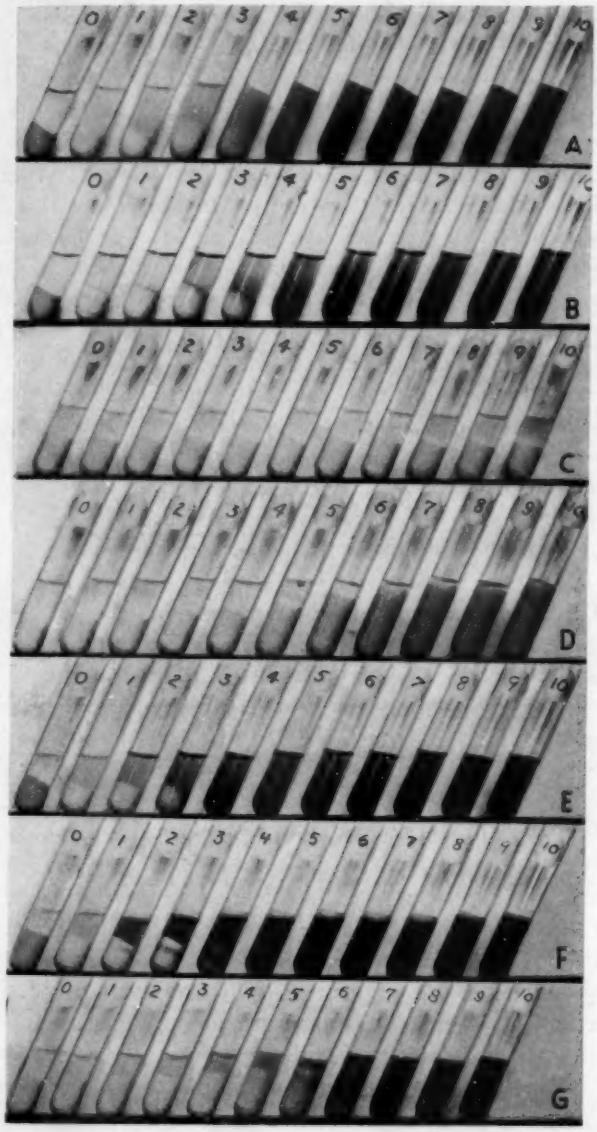
The experiment shown in *B* in the illustration brings out the same effects for an alkali, ammonium hydroxide. On comparison with *A*, it is noted that a region of swelling can hardly be discovered, the casein at once passing into solution from tube *4* on.

The effects of adding equal volumes (5 cc.) of increasingly stronger solutions of an alkyl amine, methylamine (again in this experiment and in all others referred to hereinafter, in decimal increments from hundredth-molar to tenth-molar), to a given weight of casein (0.625 Gm.) were identical with the effects of ammonium hydroxide, shown in *B*. An increase in swelling was again hardly noticeable, but the degree of solution increased steadily until in the final tube an apparently homogeneous mixture (readily miscible with any amount of added water) was obtained. This was still a liquid and hydrated colloid, however, for in spite of its optical homogeneity it was as viscid as thick glue.

An identical statement may be made regarding the primary ethyl, propyl and butyl amines as well as for the secondary methyl, ethyl and propyl amines and the tertiary methyl and ethyl amines. It may also be said of the quaternary tetramethylammonium hydroxide. As the series is ascended (either as to the complexity of the alkyl group or as to the number of hydrogen atoms replaced) the amines become increasingly insoluble in water. In the instance of such compounds as dibutylamine or tributylamine, therefore, the solubility in water will not allow of the preparation of such concentrations as are represented in the tubes shown in *B*. Nevertheless, when the proper amounts of these amines were mixed with the protein and water the ultimate result (attained in three days instead of one) was the same. We attribute this to the fact that with sufficient time even water-insoluble amines combine with the protein (as do the water-soluble amines) and the resulting compounds then swell and dissolve as do the lower members of the series. The biologic significance of this fact will be emphasized.

The amine grouping produces its effect regardless of whether it exists as free ammonia or as the primary, secondary or tertiary substitution product thereof. But when these compounds are united with acid their effects on protein disappear. Thus, when ammonium chloride is used instead of ammonium hydroxide the protein is left unaffected; and the same is true of the amines listed, the hydrochlorides of which are all without action, as shown in *C* of the illustration (methylamine hydrochloride).

When the passage is made from the alkyl amines just listed to the aryl amines, namely, phenylamine (aniline), tolylamine (toluidine), xylylamine (xylidine), naphthylamine and ethylphenylamine, both the



The colloid-chemical changes induced in unit weights of casein in the direction of its increased swelling, softening, optical clarity and solution. The successive series show the effects of standard concentrations and amounts of hydrochloric acid (*A*), ammonium hydroxide (*B*), the salt of an amine, methylamine hydrochloride (*C*), aspartic acid (*D*), paraphenylenediamine (*E*), benzylamine (*F*), and pyridine (*G*).

swelling and the solution effects on the casein, while still present, are greatly reduced. When the compounds are converted into the hydrochlorides even this slight action disappears.

Change of the alkyl or aryl amines to their corresponding hydroxylalkyl or hydroxylaryl amines leaves their original activity unimpaired.[7] But if the oxidation of the modifying hydrocarbon group is carried further, as to the aldehyde stage (instanced by the carbonyl amine commonly called formamide) or through this to the carboxyl stage (thus yielding the type picture of an amino-acid), all swelling and solvent properties disappear. From a chemical point of view, this neutral action is explained, perhaps, by the fact that a free amine group is balanced by a carboxyl group. The monocarboxyl alkyl amines, such as monocarboxylmethylamine (glycocoll), monocarboxylethylamine (alanine), monocarboxylpropylamine, monocarboxylisobutylamine (leucine), monocarboxylheptylamine and 1-carboxyl 2-hydroxylphenylethylamine (tyrosine), are without effect on casein. Several of the members of this class are the amino-acids split from protein when normally digested. Reasoning biologically, one would not expect that such compounds, constituting as they do a portion of man's daily food, would manifest any toxic properties as judged by the effects of amines on the hydration or liquefaction of a native protein; and they do not. Some support for this point of view may be found in the action of aspartic acid. This produces marked swelling of casein, as seen in *D* of the illustration. Chemically, this 1-2-dicarboxylethylamine has its one amine group offset by two carboxyl groups (and the more definitely acid nature of the compound comes into play). While aspartic acid is frequently listed as one of the normal digestion products of protein, it seems probable that the real form in which it is broken out of protein is as its amide, namely, asparagine. And asparagine is without effect on casein.[8]

The inactivity of the monocarboxyl alkyl amines is maintained even when the NH_2 group is made to replace an OH in the carboxyl group instead of an H on one of the carbon atoms of the mother acid (as in the normal amino-acids that may be split out of protein). We thus found the amides of formic, acetic, propionic, lactic, valeric, stearic, aspartic and benzoic acids to be without action. Urea, which in higher concentration produces both swelling and liquefaction of casein, appears at first sight an exception to this rule, for it is ordinarily classed as the

7. When the oxygen is inserted between the alkyl and amino groups, however, these hydrating effects disappear. Alphamethylhydroxylamine, for example, is without action, whereas its isomer, hydroxylmethylamine, is strongly active.

8. When only one of the carboxyl groups of aspartic acid is neutralized with ammonia, its swelling effect disappears (because the acid nature of the compound is reduced), to reappear when both groups are neutralized (for the balance of the compound is now on the amine side). Glutamic acid, which is 1-3-dicarboxylpropylamine, acts like aspartic acid.

diamide of carbonic acid. However, it may perhaps be looked on more correctly as aminoformamide, and its hydrating and solvent action may be dependent on the maintenance of the basic amine properties in the compound as thus conceived.

What amounts to the activation of an otherwise inert compound is illustrated in the effects of decarboxylation on histidine. Histidine, which is without effect on casein, becomes strongly active as soon as it is changed to histamine. This chemical degradation has its analog in the decarboxylation of any of the alpha amino-acids, such as the changing of glycine to methylamine or of alanine to ethylamine.

Change from a mono-amine to a diamine increases the hydration and solution effects of the compound on protein. Thus hydrazine and methylenediamine are more active than the corresponding mono-amines (ammonia and methylamine). This action appears even in the aryl amines. While the phenylmono-amines are without marked action, the phenylenediamines are very active. But in these the position of the amino groups is of great importance. While orthophenylenediamine produces some swelling and solution of casein this effect is small in comparison with that of metaphenylenediamine. The tremendous action of paraphenylenediamine is indicated in *E* of the illustration, an observation which explains, through direct action on protoplasm and without recourse to vascular change, the long known and almost specific pharmacologic action of this compound in the production of fatal edema in and about the neck.

When an aryl group is added to an active alkyl amine, as in benzylamine, the new compound retains the activity of the old (*F* in illustration). In this instance the benzene group replaces hydrogen on the carbon of the alkyl group. If it is tied to the nitrogen of the amine group (to yield the chemical isomer, toluidine) this activity practically disappears. The same is true of that other chemical isomer methylaniline.

We have also experimented with a series of nitrogenous compounds, other than those already mentioned, which are of interest because of their chemical relation to these compounds or because they are derived from the chemical nucleus of toxins, various protein-split products, including the ptomaines, or alkaloids. Thus we found pyridine (*G* in illustration), quinoline, creatinine and hexamethylenetetramine to produce swelling and solution of casein. Uric, hippuric and barbituric acids, on the other hand, were found to be without effect, as were also creatine hydrate, pyrrole, l-prolin, acetoxime, ethyl nitrite and ethyl nitrate.

COMMENT

In an attempt to explain what an acid or an alkali does to a protein to make it swell or dissolve, it was early pointed out that this has nothing to do with the hydrogen or hydroxyl ion concentrations observable in

the protein-water mixtures. The effect produced is dependent on compound formation. The proteins behave as do water-insoluble aminofatty acids or water-insoluble fatty acids. When on treatment with an alkali, for example, the latter are made to absorb more water or to pass into solution, one says not that this is because of some mysterious change in hydrogen or hydroxyl ion concentration but that the fatty acids unite with the alkali to yield a soap. Similarly, the aminofatty acids in uniting with alkali yield a soaplike compound. In both instances the newly formed products differ from the original aminofatty acid or the fatty acid in two ways—they have a greater capacity *for* dissolving water (swelling) and a greater solubility *in* water. The same action and the same effects may be observed on neutral protein when treated with alkali (as illustrated in the description of the action of ammonia on casein). We believe that the action of the amines here discussed is similar. They too, combine with the casein to yield a series of chemical derivatives which have a greater hydration capacity and a greater solubility in water. On this account they swell, become optically more homogeneous, soften or even liquefy.

We wish now to utilize these colloid-chemical findings in an attempt to make more precise the mechanism and the chemistry of that effect of injury which in physiology is expressed by the term "reaction to injury" and in pathology, by "inflammation." To say that the product of such injury consists of swelling, cloudy swelling, softening, liquefaction, leukocytic infiltration and recovery, absorption or necrosis is to approach such an analysis, but it is an analysis still in the terms of biology. A more finite explanation would lie in the substitution of physical or chemical concepts for these terms.

The essence of inflammation receives a different definition depending on whether one asks a general [9] or a special [10] pathologist; and even in the latter case it makes a difference whether the answer is made from observation on avascular or on vascular tissue. Of the total set of changes marking the reaction to injury in vascular tissue several disappear as soon as nonvascular areas or plant structures are involved, for example, changes within blood vessels, increased redness and increased heat (the fraction due to the increased circulation of blood in a warm-blooded animal). Leukocytic infiltration obviously disappears in organisms that have no leukocytes and in plants. Pain is a criterion only in sensible organisms. Yet the morphologic essence of the reaction to injury persists throughout the organized world, for, as Kite [11] showed clearly, even the individual cell when stabbed with a glass needle shows

9. Ransom, W. H.: The Inflammation Idea in General Pathology, London, Williams & Norgate, 1905.

10. Adami, J. G.: Inflammation, New York, The Macmillan Company, 1907.

11. Kite, G. L.: Biol. Bull. **25**:5, 1913; Am. J. Physiol. **32**:146, 1913.

a streak of swelling along the path of injury. In other words, the only universally present manifestations of the inflammatory response are swelling, cloudy swelling and softening or liquefaction of the involved part, to be followed by a reversal of these changes (in other words, recovery) or absorption, with or without intervening necrosis. The physiologic element of disturbed function appears both in avascular and in vascular regions. What needs emphasis is that it appears in the latter before any of the definitely vascular reactions of inflammation have taken place and that it may show itself in the avascular tissue of animals or in plants before any morphologic change is discoverable. An organ (such as any of the secreting glands or a muscle) may, in fact, be functionally dead without macroscopic or microscopic alteration. Put another way, a tissue may have lost all its functional capacity without evidence of any more visible change than is covered by the items of swelling, or cloudy swelling, with softening and liquefaction.

In the experiments detailed we have tried to indicate the chemical nature of these changes. They show how materials (other than acids) produced in the death of tissues or through the action of micro-organisms on living tissues act directly on proteins (in our experiments on the globulin half of the total proteins found in all cells) to alter their character in the direction of (*a*) swelling and (*b*) softening, liquefaction and solution.

Every attempt to analyze in chemical terms the effects of mechanical, thermal or electrical injury, or those of injury through parasitism, shows that materials of an amine nature are produced. This is true not only of the toxins (which are kept in a separate group not so much because of a biologic difference in poisonous effect as because of the type of chemical carrier to which they are bound) but especially of the toxic products of protein cleavage. Beginning with the bases produced in protein putrefaction and called ptomaines by Selmi, it was not long before Nencki isolated phenylethylamine from the group and Brieger, a whole set of mono-amines and diamines. This author achieved also the isolation of choline (hydroxyethyl trimethyl ammonium hydroxide, the degradation product of lecithin),[12] its next of kin, muscarine, and methylguanadine. More directly germane to this subject of the nature of the colloid-chemical changes observable in infection are such analyses as those of Emmerling.[13] From 860 Gm. of egg white inoculated with Staphylococcus aureus (besides more than 16.7 Gm. of various acids) he isolated 3.5 Gm. of trimethylamine; and from 600 Gm. of moist wheat gluten inoculated with Proteus vulgaris, 5 Gm. of ammonia and "appreciable quantities" of trimethylamine. This high ammonia value is largely

12. The colloid-chemical activity of the closely related tetramethylammonium hydroxide should be recalled.

13. Emmerling, O.: Ber. d. deutsch. chem. Gesellsch. **29**:2721, 1896.

the analytic product of amines. More recently Koessler and Hanke [14] proved the direct production of histamine from histidine in selected cultures of the colon bacillus. Amplification of the earlier studies of the chemical effects of sterile injury to tissue or of putrefaction or infection was brought by Vaughan and Novy [15] and Vaughan, Vaughan and Vaughan.[16] About the same time Eustis [17] showed the application of histamine (beta-iminazolylethylamine) to the slightly broken skin to be followed by intense urticaria, and Johnston [18] demonstrated the edema-producing effects of this compound and of various other amines on different tissues directly. Within recent years various amines have been discovered as the active producers of edema in sterile "wound" shock, in eclampsia and in several types of human infection—in short, in a whole series of pathologic states of which edema is the sole morphologic attribute.

The experiments detailed show what is the point of attack of such compounds. They act on the protein of the living cell and, uniting with it, yield a protein derivative. It does not matter, of course, whether at the point of injury the poisonous agent is produced immediately, as the product of protoplasmic degradation, or mediately, through the action of a pathogenic organism. In either case the protein derivative has a solvent capacity greater than normal for water and in water, and the colloid-chemical and the clinical or postmortem terms that describe the newly formed system are covered by swelling, softening, liquefaction and solution.

SUMMARY

Quantitative experiments are described illustrating the effects of a long series of different amines on the swelling, softening and solution of a pure protein (casein). The effects are attributed to compound formation in consequence of which proteinates possessed of a higher degree of solvent power for water and in water are produced. The identical compounds, elaborated in consequence of injury and of infection, are held to act similarly on protoplasm, through which an explanation in colloid-chemical terms is found for the swelling, softening and liquefaction of tissue characteristic of various pathologic states.

14. A number of papers by these authors appeared in the Journal of Biological Chemistry from 1919 to 1924, several of which deal specificially with the direct production of histamine from histidine (J. Biol. Chem. **39**:521 and 539, 1919; **50**: 131, 1922; **59**:803, 1924).

15. Vaughan, V. C., and Novy, F. J.: Ptomains, Leucomains, Toxins and Antitoxins, Philadelphia, Lea Brothers & Co., 1896.

16. Vaughan, V. C.; Vaughan, V. C., Jr., and Vaughan, J. W.: Protein Split Products in Relation to Immunity and Disease, Philadelphia, Lea & Febiger, 1913.

17. Eustis, Allan: Am. J. M. Sc. **143**:862, 1912; New Orleans M. & S. J. **66**:730, 1914.

18. Johnston, A. R.: J. Infect. Dis. **42**:473, 1928.

CHANGES IN THE MYOCARDIUM OF RABBITS FROM AUGMENTING THE HEART RATE MECHANICALLY AND FROM INDUCED HYPERTHYROIDISM

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In a previous experimental study of the heart in hyperthyroidism [1] we discussed the various opinions concerning the lesions in human hearts in association with hyperthyroidism as well as the experimental evidence of the character of such pathologic disturbances in the hearts of animals used for experimental study. We concluded that the evidence of the existence of a specific thyroxine-induced cardiac lesion was not entirely convincing.

It was further suggested that such damage to the cardiac musculature might be the result of cardiac overwork resulting from hyperthyroidism rather than due to the direct impingement of thyroxine. This opinion was based on the occurrence of similar lesions in the heart of a rabbit in which the inhibitory nerve control to the heart was destroyed. This prompted us to investigate further the rôle of uncontrolled heart action in the production of the lesions in the heart in hyperthyroidism.

Accordingly, we selected a number of healthy rabbits, divided them into four groups and placed the animals in individual cages under clean hygienic conditions on a diet sufficient to maintain a relatively constant weight. Group 1 consisted of rabbits in which the depressor nervous mechanism of the heart was destroyed. Group 2 was composed of rabbits fed desiccated thyroid (Armour's) to the point of toxicity. Group 3 consisted of rabbits with the depressor mechanism destroyed, with the additional administration of ephedrine or desiccated thyroid or

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1. Menne, F. R.; Keane, R. H.; Henry, R. T., and Jones, N. W.: Am. Heart J. **8**:75, 1932.

both. Group 4 consisted of rabbits under similar conditions of housing, but unmolested in any way. The following results were obtained:

EXPERIMENTS AND RESULTS

Group 1.—Ten rabbits were operated on under ether anesthesia. A number of these were given preliminary doses of sodium amytal but this was discontinued because of the undesirably long postoperative anesthesia resulting in respiratory congestion. The carotid canals were exposed through a midline incision, and an inch section of the depressor fibers on either side was removed. The carotid arteries were then carefully denuded of their investments up to and including the carotid sinus at the bifurcation forming the internal and external branches. Following this effacement, the denuded artery was gently swabbed with 95 per cent alcohol. An immediate enlargement of the arteries and an increase in the pulse rate were observed. Following the operation the animals were kept in a warming chamber until they completely recovered from the anesthesia.

All pulse rates were determined from the electrocardiographic tracings (lead II needles inserted beneath the skin were used for electrodes). A number of such tracings were made prior to operation in order to accustom the rabbits to being tied down and to determine the normal for a given rabbit. All such manipulation was accomplished with the minimum excitement in a quiet room. The normal pulse rate in this group varied from 175 to 330, the average being 251 per minute. The rabbits were kept under observation for a variable period from two to fifty-one days. Three of these animals showed a loss of weight; in two there existed a secondary infection. In seven of the ten rabbits an average increase in pulse rate of 63 beats per minute was obtained, while two actually showed a decreased pulse rate.

Although the electrocardiographic tracings were made with lead II, only certain changes were manifest in the action of the heart. There was evident a variation from simple tachycardia to inverted P and T waves with an occasionally altered Q R S complex. In rabbit 29 there was an additional auricular fibrillation. These findings were not altogether constant or in proportion to the extent of the myocardial changes found at autopsy. They were, however, in general, suggestive of myocardial damage.

Seven of the rabbits were killed at the end of the observation period. Three were found dead. All were carefully studied at autopsy. Search was made for such disturbances as might have a bearing on the changes in the heart. In six of the rabbits, no noteworthy changes were found. In two there were stitch abscesses, while in two others abscesses were found in the neck and left lung, respectively.

Gross Examination of the Hearts: A moderate variation was observed in the gross appearance of the hearts (fig. 1). In general, however, the auricles were found moderately dilated. The subepicardial fat of the ventricles was scant. The left ventricles appeared elongated and hypertrophic. The hearts of the animals found dead were dilated, being filled with blood. The average weight of the hearts of the animals that were killed was 8.9 Gm. as compared with 12.2 Gm. in the rabbits that died, the difference being in part due to the blood content in the latter. The heaviest hearts were found in those animals in which infections were found and also in one in which there was an ileus due to cutting of the right vagus nerve. The smaller hearts were found in the rabbits that were observed the longest after operation.

The average length of the ventricles from the auriculoventricular groove to the apex was 3.4 cm., being the same in the animals that were killed and those that died. A variation occurred in the transverse diameters, the average in the animals that were killed being 2.44 cm. as compared with 2.76 cm. in those found

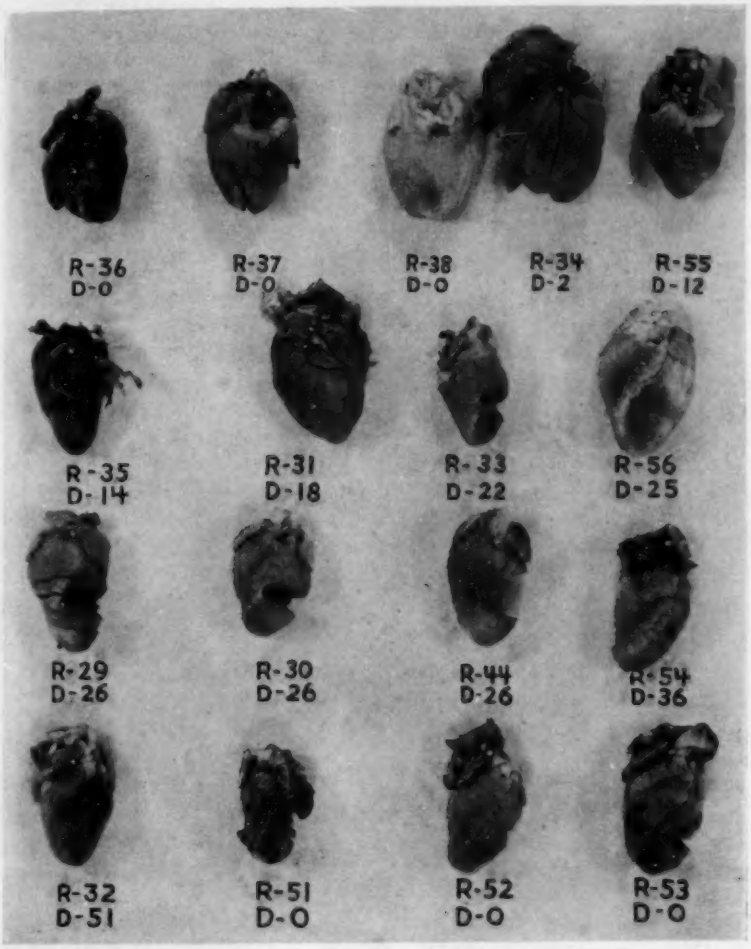

Fig. 1.—Photomicrograph showing the gross appearance of the hearts in group 1. Portions were excised promptly after weighing so that they might be fixed for the purpose of demonstrating glycogen.

dead. This difference was most marked at the apex (1 cm. from the tip) where the hearts of the animals that were killed measured 1.61 cm. as compared with 2.23 cm. in those that died. Evidently cardiac dilatation affected the transverse measurements to a greater extent than it did the lengths of the hearts. When the hearts were sectioned transversely, the reverse was found in the measurements of the left ventricles. In the animals that were killed the left ventricles averaged 1.8

cm. as compared with 1.6 cm. in the rabbits found dead. Hypertrophy of the wall was found to be responsible for this difference.

Microscopic Examination: Sections were made at three different levels through both ventricles. An attempt was made to determine the glycogen content by the use of special stains. Such efforts were unsuccessful. Because of an inability to demonstrate glycogen with such methods in control animals, the effort was abandoned. The glycogen in the cardiac muscle evidently exists in such a nebulous state that it is not readily detectable by staining methods. All sections were stained with hematoxylin and eosin and with phosphotungstic acid-hematoxylin. Relatively bizarre cytologic changes were observed. There was

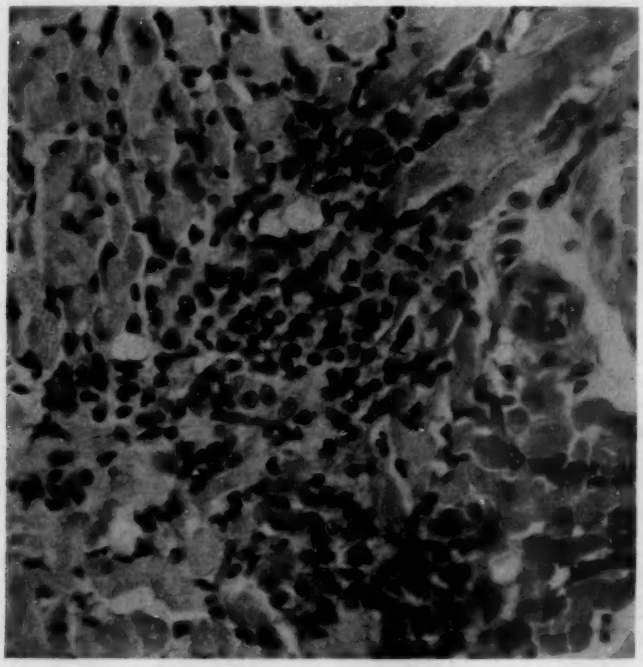

Fig. 2 (group 1).—Photomicrograph of an inflammatory-like intermuscular accumulation of histiocytes. There was no gross evidence of infection in this animal.

noticed an irregularity in staining. Even with the most careful technic certain areas failed to be differentiated. Here the muscle bundles appeared swollen, and the cross-striations were found to be blurred. Then, too, small collections of histiocytes (monocytes) would be seen to separate the muscle bundles (fig. 2). In still other areas distortion of muscle bundles with fraying, small fat vacuole formations and early hyperplasia of the fibrous connective tissue were noted (fig. 3), while in still other areas the fibrosis and wide separation of the muscle bundles were more extensive (figs. 4 and 5). These illustrations are all from the heart of rabbit 29. This animal was allowed to survive twenty-six days after operation. The course, with the exception of the increased heart rate and the general autopsy observations, was entirely normal. The lesions found in the

papillary muscles, particularly those supporting the chordae tendineae of the mitral valve, were found to be older, more hyaline and more extensive. On the basis of such abnormal histologic changes, examination of two of the hearts of the rabbits gave negative results while the results in five were considered slightly positive and in three definitely positive.

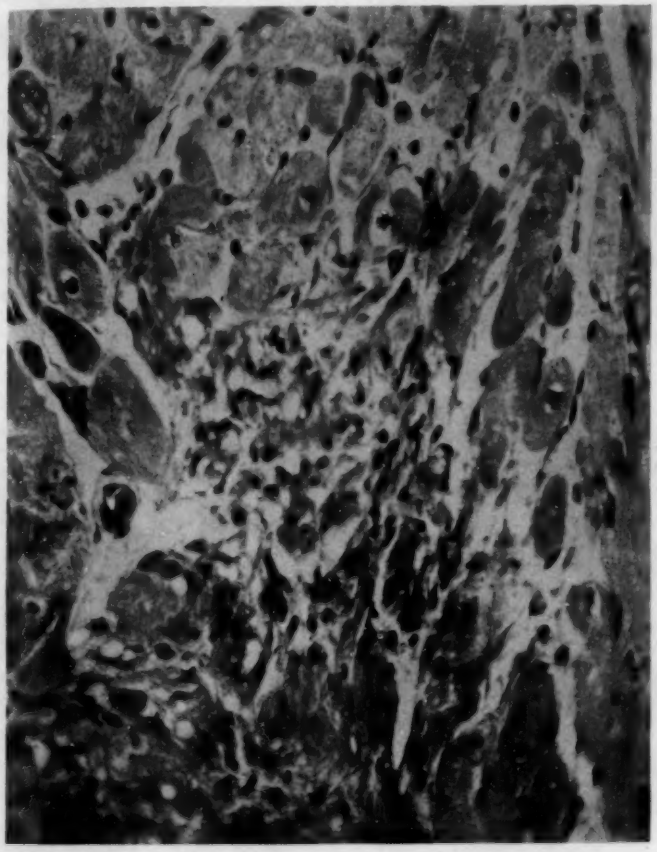

Fig. 3 (group 1).—Photomicrograph of an early focal degeneration of the myocardium. Note the vacuole formation and distortion of the continuity and relationship of the muscle bundles.

Comment.—In arriving at a conclusion as to the significance of the changes occurring in the hearts of rabbits with the depressor nerve mechanism to the heart destroyed, several important factors must be taken into consideration. First, the completeness of the operative intervention will be considered. Although it seems relatively simple, certain difficulties were encountered. The heart rate was, on the average, very rapid. In certain animals in which the heart rate was high, it was often

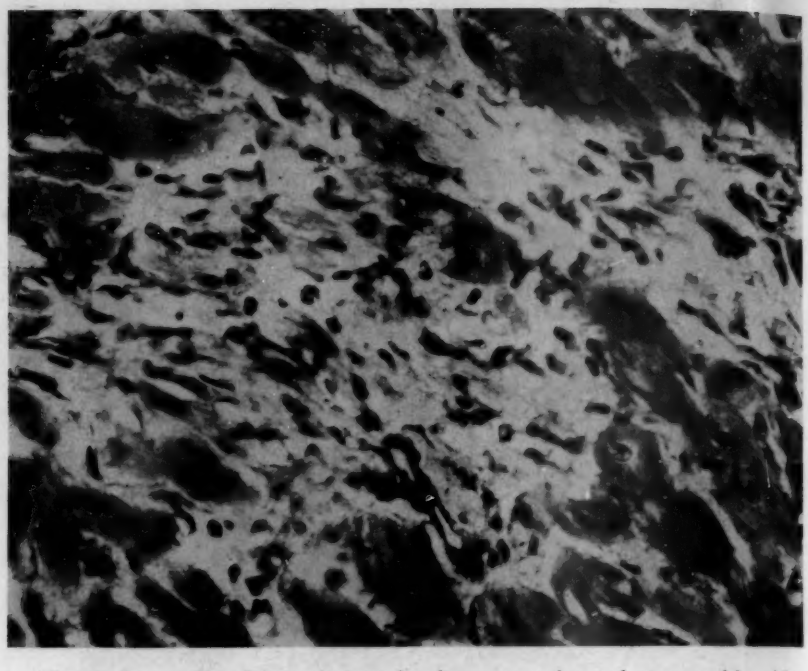

Fig. 4 (group 1).—Photomicrograph of a more advanced stage, with wider separation of muscle bundles and fraying, owing to focal fibrosis.

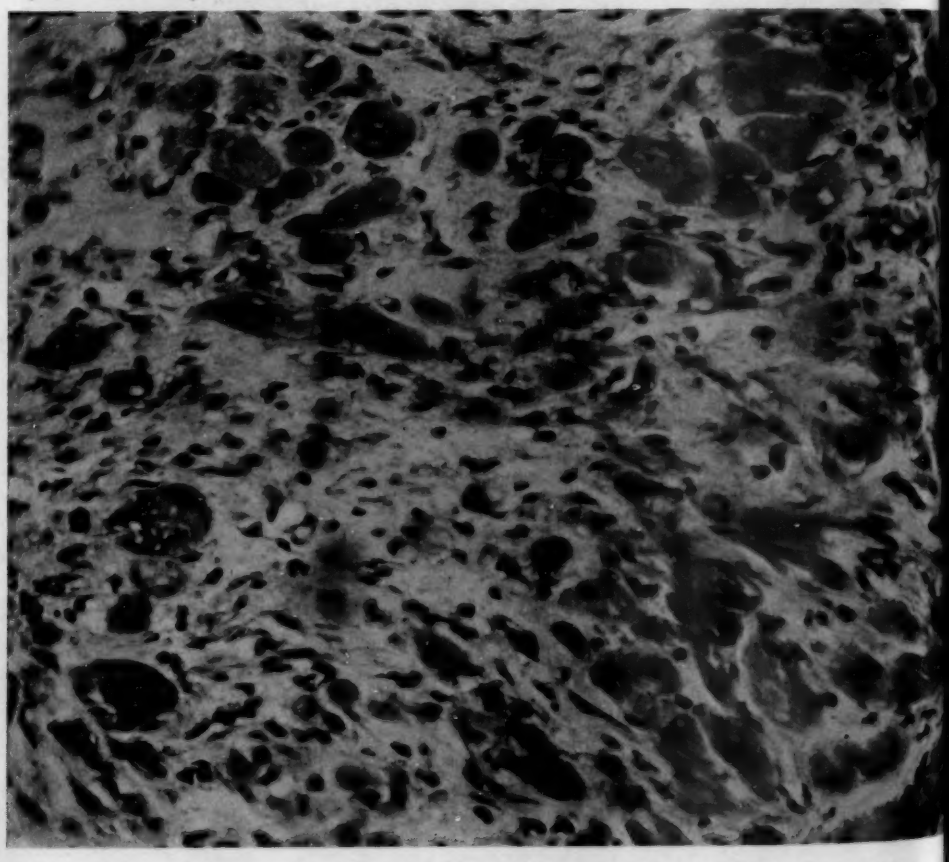

Fig. 5 (group 1).—Photomicrograph of an area of marked fibrous connective tissue separation and replacement of muscle bundles.

difficult to increase the rate by operation. Then, too, accessory anastomosing nerve fibers may and probably do occur, so that the inhibition was only in part destroyed, and this was apparently compensated for. In a number of instances, two or three depressor (?) fibers were found accompanying the vagus. Secondly, one must consider the possibility of individual variation in the compensatory adjustment of the cardiac activity, and thirdly, the fact that the animal was confined to its cage and not in its natural wide range habitat, where ordinary vicissitudes would influence the strain placed on its irritable heart, must be reckoned with. In addition to these the possibility of preexisting damage to the cardiac muscle must also be considered. There is also no way of determining many of the disturbances that may call for excessive cardiac strain such as occur in human beings. In this experiment there seems to be some relationship between the duration and the extent of the lesions. It would seem that they appeared most strikingly in the first three weeks, since one of the rabbits with a twenty-six day course showed more definite lesions than were found in the rabbit living fifty-one days after the operation.

There appears to be a parallelism of the increase of the heart rate and the occurrence of lesions within the heart. The more pronouncedly damaged hearts were found, with one exception (rabbit 30), in the rabbits in which the heart rate was increased above 60 beats per minute. Individual compensatory adjustment may account for the lack of exact gradation of the lesions. Most of these animals were observed to be inactive. They were found lying down a great deal of the time. This undoubtedly had the effect of lessening the strain on the heart. The temperament of the individual animals must be considered in allowing for such compensatory adjustments.

The rôle of infections in the production of the pathologic changes in the hearts is of interest, since it may be argued that they are the causative agents. Three of the rabbits in this series had infections. One of these died with a stitch abscess, but no lesions were found in the heart. In the other two, which had distinctive changes in the heart, such lesions were not considered more pronounced than those in the hearts of the animals that had no traceable infection. In none of the hearts of the infected animals was there evidence of mycotic foci. Polymorphonuclear leukocytic infiltration was not found, and yet one cannot exclude the possibility of circulating toxins in such instances. We were unable, therefore, to conclude that such lesions were produced by the coexisting infections, although we must admit their augmenting rôle.

There appears to us to be a definite relationship of the lesions in the hearts of the rabbits of this group to the altered increased rate of the heart induced by destroying as completely as possible the inhibitory nerve mechanism.

TABLE 1 (Group 1).—*Experimental Data Concerning Rabbits with Cardiac Inhibition Destroyed*

	Experimental Data						Data on Heart						
Number	Weight, Kg.	Pulse	Number of Days	Weight Loss, Kg.	Pulse Increase	Death*	Weight, Gm.	Length, Cm.	Width at Base, Cm.	Width at Apex, Cm.	Width of Left Ventricle, Cm.	Results	Comment
29	2.39	240	26	0	60	A	8.2	4.0	2.5	1.6	1.7	Positive +++	Observation: Negative course Autopsy: Negative; heart: gross—pale, right auricle dilated, elongated; microscopic—atrophy, hypertension, fibrosis, fraying
30	2.50	225	26	0	75	A	7.0	3.0	2.0	1.4	1.75	Positive +	Observation: Negative course Autopsy: Negative; heart: gross—broad at base, right chambers dilated, petechial hemorrhage in myocardium; microscopic—cell infiltration, fraying, atrophy, hypertension
31	2.16	240	18	0	45	D	15.9	3.5	2.8	2.5	1.7	Negative	Observation: Stitch abscess; died Autopsy: Abscess in neck; general parenchymatous degeneration; heart: gross—right auricle dilated; microscopic—parenchymatous degeneration, edema, fragmentation
32	2.39	240	51	0	48	A	8.7	3.2	2.3	1.5	1.8	Positive +	Observation: Negative course; left carotid artery ligated Autopsy: Negative; heart: gross—firm, moderately elongated, auricles dilated; microscopic—focal atrophy and hypertension, fatty degeneration, fraying, fibrosis
33	2.27	330	22	0	—30	A	5.7	3.1	2.0	1.4	1.5	Negative	Observation: Negative course Autopsy: Negative; heart: gross—atrophy, narrow, elongated; microscopic—focal atrophy and hypertension, hyaline degeneration, slight fibrosis

34	2.61	300	2	0	—42	D	11.0	3.8	3.2	2.5	1.8	Positive +	Observation: Right vagus cut at operation; spasmodic respiration; diarrhea; found dead Autopsy: Emphysema, congested pleural cavity, dilatation of stomach and bowel; heart: gross—marked dilation; microscopic—atrophy, fragmented, fraying, fibrosis, hyaline degeneration
35	2.30	240	14	0.56	60	D	9.7	2.9	2.3	1.7	1.3	Positive ++++	Observation: Stitch abscess; found dead Autopsy: Abscess in neck, congested pleural cavity; heart: gross—dilation, small at point of apex; microscopic—atrophy, hypertension, fatty and hyaline degeneration, cellular infiltration, fibrosis, fraying
54	2.08	320	35	0	0	A	10.4	3.5	2.4	1.7	1.7	Positive +	Observation: Negative course; gave birth to 2 rabbits Autopsy: Negative; heart: gross—elongated, hypertension, left ventricle pale; microscopic—focal hypertension and atrophy, hyaline degeneration, fibrosis
55	3.80	200	12	0.53	66	A	10.85	3.4	2.9	1.9	2.1	Positive ++	Observation: Negative course Autopsy: Negative; heart: gross—dilated, broad base, pointed apex; microscopic—focal atrophy and hypertension, cellular infiltration, fraying, fibrosis, hyaline degeneration
56	3.87	175	25	0.20	89	A	11.7	3.6	3.0	1.8	2.1	Positive +++	Observation: Negative course Autopsy: Small abscess of left lower lobe; heart: gross—pale, broad base; microscopic—focal edema and atrophy, fibrosis and fraying
Average	2.63	251	..	....	7R, 63+ 3R, 24—	..	A, 8.9 D, 12.2	A, 3.4 D, 3.4	A, 2.44 D, 2.76	A, 1.61 D, 2.23	A, 1.8 D, 1.6	Positive 8 Negative 2	

* In this and the following tables, A indicates animals killed, and D, those that died.

In order to study further the comparative effects of unbridled action of the heart, mechanically induced, with that of the rapid heart rate of rabbits with induced hyperthyroidism, we decided to investigate a somewhat similar series of such animals under the same conditions.

Group 2.—The rabbits were about the same average weight as those in group 1. They were raised, housed and fed in the same manner. They were given doses of desiccated thyroid (Armour's) in amounts varying from 90 to

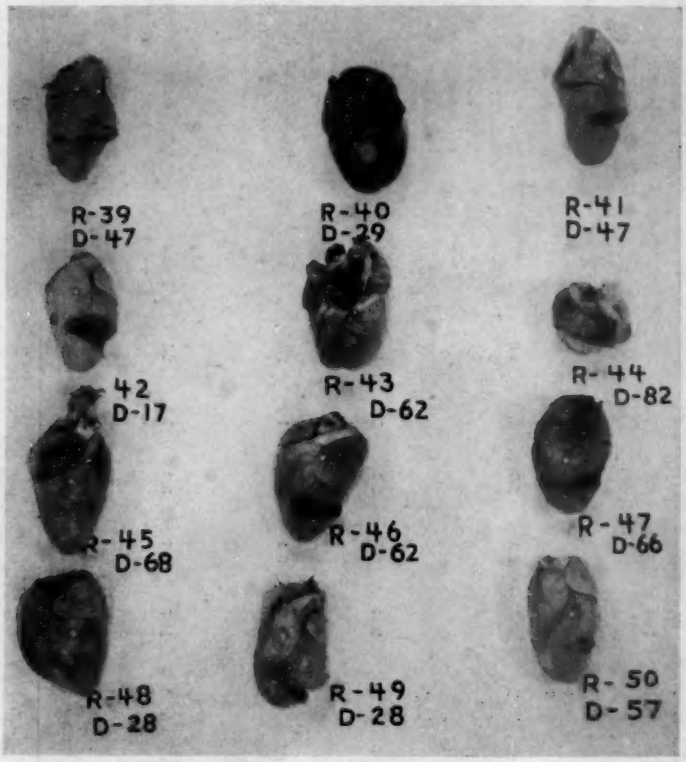

Fig. 6 (group 2).—Photograph of the gross appearance of the hearts of rabbits with induced hyperthyroidism.

720 mg. daily in two dose-periods of varying days with various intermissions, the object of this procedure being to induce the animal to pass through two successive toxic periods. Considerable variation was encountered as to the tolerance of rabbits toward desiccated thyroid. Some of them became rapidly toxic on 90 mg. of thyroid a day, while others increased in weight, became complacent and even somewhat lethargic. In four instances it became necessary to increase the dosage to from 270 to 720 mg. daily before the toxic symptoms began to appear. The toxic dosage was continued until marked loss of weight, increased irritability and weakness developed. It was then discontinued until the animals began to approach

a normal status, when a second series of feeding was started and carried to a similar end-point. Each animal, therefore, received two rather distinctive jolts.

The average increase in pulse rate (taken by electrocardiographic tracings) ranged from 30 to 165 beats per minute, the average for the group being 82. The

Fig. 7 (group 2).—Photomicrograph of the myocardial damage with fat vacuole formation, fraying of muscle bundles and early fibrosis.

majority of the rates were high, since the average also includes those slower rates occurring when the animal was approaching collapse.

The electrocardiographic tracings (lead II) revealed a marked variation from inverted P and T waves to regular or slower or normal rhythm, while in others the reverse was true. Rabbit 50 showed an inverted P wave occurring at irregular

times. In rabbit 48 there was a regular to irregular rhythm with sinus block. In rabbit 43 the rhythm was regular to irregular with final flutter. The latter occurred in three rabbits (43, 46 and 47). These changes also point to myocardial damage. Again the variations in rhythm were not entirely consistent with the modifications of the cardiac muscle found at autopsy. Six of these rabbits were killed when they were near collapse, while five died during the night.

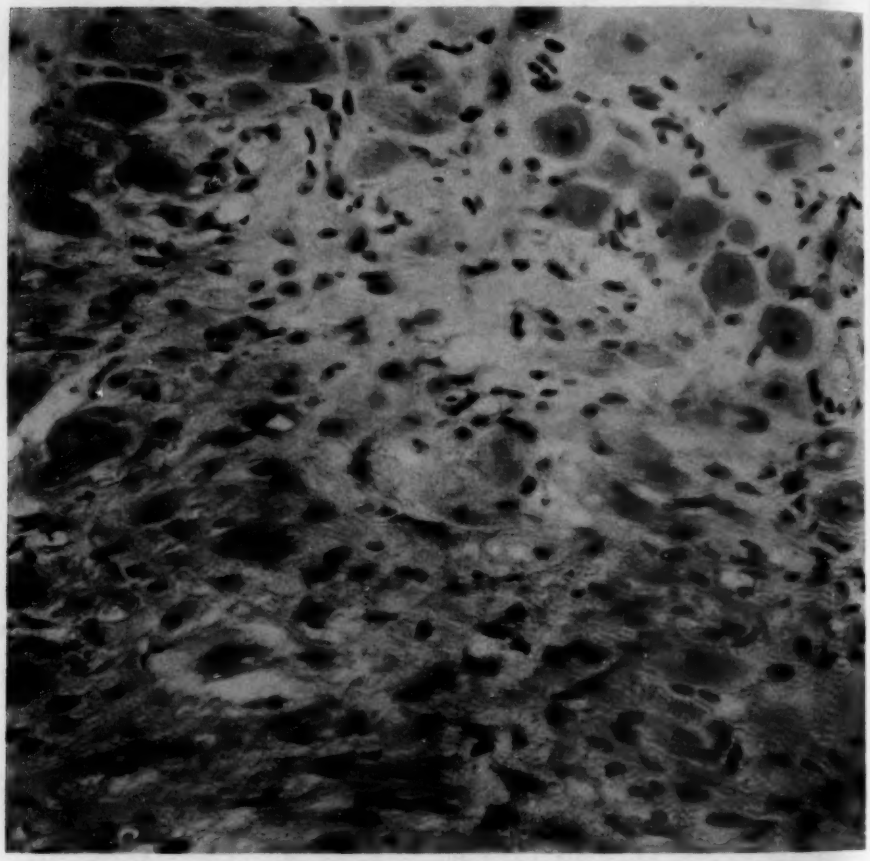

Fig. 8 (group 2).—Photomicrograph showing extensive degeneration and separation of the muscle bundles with fibrous connective tissue replacement.

Autopsy revealed general emaciation in all, and in two (43 and 48) abscesses were found in the right pleural cavities. No other noteworthy gross pathologic changes were found.

Gross Examination of the Hearts: All of the hearts were practically devoid of subepicardial fat (fig. 6). Four of the five hearts of the rabbits that were found dead showed marked right-sided dilatation. In general, the hearts of the rabbits that were killed were pale, firm and elongated, the average weight being 8.16 Gm. As may be seen in table 2, all measurements were reduced, with such variations as might be expected in dilated hearts.

Microscopic Examination: As was true in the examination of the hearts in group 1, a similar but more extensive polychromatophilia was noted. Regional shrinking and focal hypertrophy of the muscle bundles were observed. A spotty swelling of the muscle bundles with blurring of the cross-striation was noted. In still other areas rather complete dissociation with interruption of the muscle bundles was seen (fig. 7). Here a fibrillar reticulum separated by circular fat vacuoles with fraying of the muscle bundles and early proliferation of fibroblasts was noted. As in the examination of the hearts in group 1, all gradations to larger areas of replacement of fibrous connective tissue were observed (fig. 8). Here, too, the pathologic lesions were found in the left ventricle. They appeared to be more pronounced in the papillary muscles and in the lower half of the left ventricular walls. Ten of these hearts were regarded as having definite lesions. The latter were most pronounced in the hearts of the rabbits with abscesses in the pleural cavities.

Comment.—It seems to us that there is sufficient evidence to indicate that distinctive damage to cardiac musculature occurred in these rabbits following the administration of repeated doses of desiccated thyroid. While it is true that heroic doses were administered over long periods in order to produce such disturbances, it must be remembered that the housing of the rabbits in cages is comparable to placing a patient with hyperthyroidism at rest in bed. Under such conditions there is a minimum taxation of cardiac function. The variation in the dosage employed and the total number of days required as well as the time interval between doses appear to us to be consistent with individual constitutional ability of each animal to resist, maintain and restore metabolic equilibrium. Intimately linked with this status are the loss of weight, increase in the pulse rate and the ability to resist infection and death. The weights and measurements of all the hearts in this group are proportionate to the variations in body weight, the terminal condition of the body as a whole and of the heart in particular being one of inanition. The variations in the extent of cardiac damage, as may be seen in table 2, were not always proportionate to the severity of the toxicity, since the animals that were found dead from the thyroid insult did not always reveal extensive pathologic changes in the heart. This fact may mean that our examination and criteria of cardiac disturbances were not sufficiently comprehensive or that greater influence must be attached to extracardiac failure in bringing about death. It is likely that rabbit hearts may vary in their susceptibility to injurious agents just as do human hearts. In rabbits 43 and 48, the presence of an infection (probably secondary to the lowered resistance) seems to have augmented the destruction of the cardiac muscle. There was no way of determining the exact relationship of these two conditions in these animals. The fact that there were these discrepancies in results when the body was flooded with the active principle of desiccated thyroid would seem to vitiate the contention that there is a specific differentiated effect of thyroxine on the cardiac muscle.

TABLE 2 (Group 2).—*Effect of Feeding Desiccated Thyroid to Rabbits*

	Experimental Data									Data on Heart						
Number	Weight, Kg.	Pulse	Mg. Thyroid Daily	Number of Days	Interval, Days	Amount of Thyroid Fed, Gm.	Weight, Loss, Kg.	Pulse Increase	Death	Weight, Gm.	Length, Cm.	Width at Base, Cm.	Width at Apex, Cm.	Width of Left Ventricle, Cm.	Results	Comment
50	2.12	186	90-150	56	15	7.56	1.0	72	A	9.9	3.9	2.2	1.7	1.5	Pos. +++	Observation: Irritability, loss of weight, diarrhea, weakness Autopsy: Emaciation, atrophy; heart: gross—small, shrunken, broad at base; microscopic—atrophy, focal hypertension, regeneration, fragmentation, fatty changes, fraying, fibrosis
47	2.82	180	90-180	65	15	9.18	0.97	76	A	9.5	3.3	2.5	1.9	1.7	Pos. +	Observation: Irritability, weakness, prostration Autopsy: Emaciation, congested pleural cavity; heart: gross—negative; microscopic—polychromatophilia, edema, fraying, slight fibrosis
48	2.57	300	90-180	65	15	9.18	0.94	82	D	9.9	3.5	2.8	2.6	1.8	Pos. ++++	Observation: Marked toxicity, dropped heart beat; found dead Autopsy: Abscess, right pleural cavity, extending through diaphragm; heart: gross—firm, elongated; microscopic—marked chromatophilia, fibrosis, fraying, edema, fatty change
49	2.61	155	90-180	66	15	9.36	0.46	100	A	8.45	3.2	2.5	2.2	2.0	Pos. +	Observation: Emaciation, diarrhea, weight increase during intermission Autopsy: Negative; heart: gross—small, irritable; microscopic — polychromatophilia, atrophy, hypertrophy, fatty changes, fibrosis
42	2.21	150	180	17	0	3.06	0.42	90	A	6.9	3.1	2.3	1.6	1.5	Neg.	Observation: Irritability, emaciation, exhaustion Autopsy: Negative; heart: gross—small, pale, mottled, firm; microscopic—marked polychromatophilia, focal atrophy and hypertrophy

40	2.65	210	180	17	13	3.06	0.32	70	D	11.0	3.5	2.4	1.9	1.7	Pos. +	Observation: Loss of weight, died suddenly Autopsy: Negative; heart: gross—slight hematopericardia, dilated; microscopic—polychromatophilia, focal atrophy and hypertension, slight focal fatty degeneration, fibrosis, fragmentation, fraying
39	2.61	240	180-270	47	26	9.63	0.69	60	A	7.0	3.6	2.3	1.3	1.7	Pos. +	Observation: Extreme irritability, weakness, diarrhea Autopsy: Marked emaciation; heart: gross—small, firm, left ventricle eccentric; microscopic—polychromatophilia, focal atrophy and hypertension, fragmentation
41	2.62	210	180-360	47	26	13.86	1.23	90	A	7.2	3.5	2.3	1.86	1.6	Pos. +	Observation: Increasing tolerance, final irritability, diarrhea Autopsy: Marked emaciation, congested pleural cavity; heart: gross—elongated, pale, firm; microscopic — polychromatophilia, focal atrophy and hypertension, hyaline degeneration, edema, fragmentation
46	2.27	155	180	62	9	11.16	0.91	165	D	11.47	3.6	2.7	2.0	1.7	Pos. ++	Observation: Irritability, exhaustion, found dead Autopsy: Negative; heart: gross — dilated, pointed apex; microscopic—polychromatophilia, hyaline degeneration, edema, fragmentation, fibrosis
45	2.40	285	180-270	34	9	13.59	0.61	68	D	12.8	3.4	2.3	1.6	1.7	Pos. ++	Observation: Irritability, loss of weight; died during intermission Autopsy: Congested pleural cavity; heart: gross—elongated, firm, pointed; microscopic—polychromatophilia, focal atrophy and hypertension, hyaline degeneration, cellular infiltration, fibrosis
43	2.12	210	180-720	62	26	22.14	0.72	30	D	10.0	3.4	2.6	2.1	1.6	Pos. ++++	Observation: Increasing tolerance, weight increased, final diarrhea, weakness, prostration; found dead Autopsy: Congested pleural cavity, abscess of lower lobe of right lung; heart: gross—pale, dilated; microscopic—parenchymatous degeneration and hypertension, edema, marked fibrosis
Aver.	2.4	207.3	.....	..	..	.....	0.75	82	6, A 5, D	A, 8.16 D, 11.03	A, 3.83 D, 3.48	A, 2.35 D, 2.56	A, 1.75 D, 1.65	A, 1.66 D, 1.70	Pos., 10 Neg., 1	

TABLE 3 (Group 3).—*Experimental Data Concerning Rabbits with Cardiac Inhibition Destroyed Followed by the Administration of Desiccated Thyroid and Ephedrine*

	Subgroup A								Data on Heart						
Number	Weight, Kg.	Pulse	Treatment	Number of Days	Loss, Kg.	Pulse Increase	Pulse Pressure Increase, Mm. Hg	Death	Weight, Gm.	Length, Cm.	Width at Base, Cm.	Width at Apex, Cm.	Width of Left Ventricle, Cm.	Result	Comment
44	2.05	200	Desiccated thyroid. 180 mg., 23 days post-operatively	44	0.16	+50 to −50	....	A	8.75	3.2	2.5	1.9	1.8	++++	Observation: Weight decreased, weakness, diarrhea, following thyroid administration Autopsy: Chronic fibrous pleuritis on left side, congested pleural cavity; heart: gross—elongated, pale, firm; microscopic—marked fatty and hyaline degeneration, cellular infiltration, fraying, fibrosis
65	3.12	256	Desiccated thyroid, 180 mg.; ephedrine, 3 mg. daily	25 (11)	0.88	45.0	40.5	A	8.9	3.5	2.7	1.7	1.9	++++	Observation: Loss of weight, weakness, exhaustion, following thyroid administration Autopsy: Negative; heart: gross—hypertrophied left ventricle, pale, mottled; microscopic—hypertension, cellular infiltration, fatty and hyaline degeneration, fibrosis, fraying
67	3.0	271	Desiccated thyroid, 180 mg.; ephedrine, 3 mg. daily	25 (11)	1.64	77.5	98.0	D	6.02	2.9	2.5	1.7	1.8	+	Observation: Emaciation, weakness, prostration Autopsy: Negative; heart:gross—small and firm; microscopic—atrophy, edema, hyaline and fatty degeneration, focal fibrosis
Aver.	2.72	242			0.89	40.8	69.25		A, 8.82 D, 6.02	A, 3.35 D, 2.9	A, 2.6 D, 2.5	A, 1.8 D, 1.7	A, 1.7 D, 1.8		
				Subgroup B											
61	3.9	200	Ephedrine, 4 mg. twice a day	(47) 50	0.3	32.7	68.9	A	10.2	2.7	2.6	1.8	2.0	++	Observation: Negative course Autopsy: Negative; heart: gross—large, hypertrophied; microscopic hypertension, cellular infiltration, fatty and hyaline degeneration, fibrosis
62	3.3	220	Ephedrine, 4 mg. twice a day	(19) 35	0	86.5	57.0	A	11.5	2.7	2.7	2.0	1.9	+	Observation: Negative course Autopsy: Negative; heart: gross—hypertrophied; microscopic—slight atrophy, hypertension, fatty degeneration, cellular infiltration, fibrosis
63	2.1	228	Ephedrine, 4 mg. twice a day	(28) 42	0.1	7.2	72.5	A	8.77	2.7	2.9	1.9	2.2	—	Observation: Negative course Autopsy: Negative; heart: gross—enlarged; microscopic—negative except for hypertension
64	3.5	248	Ephedrine, 4 mg. twice a day	(26) 39	0.2	20.1	57.8	A	10.0	3.1	3.0	2.0	2.0	+++	Observation: Negative course Autopsy: Negative; heart: gross—left ventricle hypertrophied, dilated; microscopic—focal atrophy and hypertension, hyaline degeneration, fibrosis, fraying, edema
Aver.	3.3	224			0.15	38.6	64.05		10.1	2.8	2.8	1.9	2.0		

Group 3 (table 3).—Since we were able to produce comparable cardiac lesions by either of the two previous experimental procedures (groups 1 and 2), it occurred to us that a combination of both of these experimental conditions should be productive of cumulative results. We therefore operated on seven rabbits. After their immediate recovery from the operation (forty-eight hours), desiccated thyroid was administered to three in daily doses of 180 mg., while two rabbits were given in addition daily intramuscular injections of 4 mg. of ephedrine. In addition, four rabbits were given 4 mg. of ephedrine twice a day but were not fed desiccated thyroid. The ephedrine was added in an attempt to increase the peripheral resistance by raising the pulse pressure and thereby increasing the work of the heart. The pulse rates and blood pressures were determined by the method of MacGregor [2] before each injection of ephedrine. The foregoing dosage was found to bring about the maximum increase in the pulse pressure in the different animals used. Although the series is small, there was found a decided variation in the response to ephedrine. The ephedrine was not administered during the entire period of observation, but only for the number of days indicated in the brackets ("No. of days," table 3).

In the animals fed desiccated thyroid, the loss in weight was found proportionate to the degree of toxicity. The increase in pulse rate was found greatest in the two rabbits receiving both desiccated thyroid and ephedrine. Although the average increase was not as great in any of these animals as it was in those of groups 2 and 3, the counter-effect of the ephedrine may have been responsible. The increase in pulse pressure was apparently somewhat greater in the rabbits (65 and 67) that received both desiccated thyroid and ephedrine. Only one of these animals (67) died of prostration.

The clinical course of these animals is in sharp contrast in the two subgroups. After several days the rabbits given desiccated thyroid became irritable, lost weight and became weak, diarrhea and prostration appearing, while the course of the rabbits receiving only ephedrine was negative.

The electrocardiographic tracings (lead II) revealed simple tachycardia in two of the rabbits (61 and 65), while in rabbit 44 there were inverted P and T waves with irregular cycle lengths indicative of dying muscle. In rabbit 44 there was also a tachycardia with inverted P and T waves with cycles of flutter. Only two tracings were made of these animals, but the findings were consistent with the damage in the hearts.

The autopsies in general gave negative results, with the exception of the occurrence of an old chronic fibrous pleuritis in one rabbit (44).

Gross Examination of the Hearts: The hearts in rabbits 44, 65 and 67 were small, pale and devoid of subepicardial fat and averaged 7.42 Gm. in weight. On the other hand, the hearts of the group of rabbits that received ephedrine were large, broad, deeply pinkish red and covered with subepicardial fat, and averaged 10.1 Gm. in weight. As is indicated in the measurements, the hearts in subgroup A were longer and narrower than those in subgroup B. In the rabbits receiving postoperative toxic doses of desiccated thyroid, a badly damaged cardiac muscle (4 plus) developed, while a similar effect was produced in only one of the two rabbits receiving in addition maximum doses of ephedrine for the same period; this variation is unexplainable, since in the second animal (67) a greater loss in weight and increase in pulse rate and pulse pressure developed, unless individual resistance is considered responsible. The results obtained in the rabbit receiv-

2. MacGregor, Leone: Arch. Path. **5**:630, 1928.

TABLE 4 (Group 4).—*Data on Controls—Normal Rabbits*

Number	Weight, Kg.	Pulse	Number of Days	Weight Loss, Kg.	Pulse Increase	Death	Data on Heart					Result	Comment
							Weight, Gm.	Length, Cm.	Width at Base, Cm.	Width at Apex, Cm.	Width of Left Ventricle, Cm.		
36	2.39	180	25	0.09	0	D	8.5	3.2	2.6	1.9	1.7	Neg.	Observation: Negative course; died at operation Autopsy:Negative; heart: gross—slightly dilated; microscopic—negative
37	2.39	240	25	0	0	D	10.1	2.9	2.7	1.6	1.7	Neg.	Observation: Negative course; died at operation Autopsy: Negative; heart: gross—dilatation; microscopic—negative
38	2.16	210	25	0.11	0	D	7.7	2.4	2.6	2.0	1.8	Neg.	Observation: Negative course; died at operation Autopsy: Negative; heart: gross—dilatation, pale; microscopic—edema, negative otherwise
51	2.20	210	68	0	0	A	7.2	2.9	2.3	1.3	1.8	Neg.	Observation: Pulse irregularity with extrasystole (constant) probable sinus block Autopsy: Negative; heart: gross—negative; microscopic, negative
52	1.52	180	68	+1.59	0	A	8.7	3.1	2.5	1.2	1.1	Neg.	Observation: Negative course Autopsy: Negative; heart: gross—pale, pointed apex, broad at base; microscopic—negative
53	2.29	180	68	+1.07	0	A	10.9	3.4	2.6	1.8	1.9	Neg.	Observation: Negative course Autopsy: Negative; heart: gross—negative; microscopic, negative
Average	2.16	200	..	....	0	..	D, 8.84 A, 8.93	D, 2.85 A, 3.13	D, 2.63 A, 2.46	D, 1.83 A, 1.43	D, 1.73 A, 1.6		

ing only ephedrine following the operation were definite, but the lesions in the heart were not as extensive as anticipated. In addition to technical difficulties, the probable preoperative hypertrophy of these hearts must be considered as mitigating the effect, since the ephedrine was administered from nineteen to forty-seven days prior to the destruction of the inhibitory mechanisms of the hearts.

Apart from these modifying influences this experiment suggests an augmented supportive rôle of destroyed cardiac nerve inhibition, the administration of desiccated thyroid and the increase of vascular tonus.

Group 4 (table 4).—This group of six rabbits constitute the normal control group. These rabbits were selected and kept under the same conditions as those in the other experimental series. They remained at a relatively constant weight except for an increase in two of them. Three died at operation. Their pulse rates remained relatively constant, averaging 200 beats per minute. One of the rabbits revealed a cardiac irregularity interpreted as a sinus block, extrasystoles appearing in the electrocardiographic tracings. Rabbits 36, 37 and 38 continued to have a normal rhythm. In rabbits 52 and 53 there were inverted P and T waves. The clinical course was otherwise negative as were the gross findings at autopsy.

Gross Examination of the Hearts: All of the hearts were considered of moderate size and rather deeply colored, with the usual distribution of subepicardial fat. Except in two instances (37 and 53), the weights were relatively constant, averaging about 8.8 Gm. The hearts of the rabbits that died were shorter and broader than those of the ones that were killed.

Microscopic Examination: None of the lesions described in the hearts of the other groups were seen.

COMPARATIVE RESULTS

A consideration of the comparative results (table 5) revealed a relative uniformity in the average original weights of the rabbits used in the different series, the one exception being those in subgroup A in group 3. There was no loss of weight in group 1, the rabbits operated on to destroy the inhibitory fibers to the heart. The greatest loss in weight occurred in the series of group 3 A, in which both the operative procedure and the administration of desiccated thyroid were used. Considering the variations in pulse rate that may be found in normal rabbits, the original average pulse rates were fairly uniform (200 to 251). The greatest increase in pulse rate occurred in rabbits fed desiccated thyroid (increase of 82 per minute). Comparable with this is the increase in pulse rate of 63 in seven of the rabbits of group 1, while in three of the rabbits decreased pulse rates actually developed. In these animals the pulse rate decreased immediately following the operation, but showed an increase later in the course of observation.

Certain irregularities occur in the weights of the hearts. Whenever autopsy was performed the large vessels leaving and entering the hearts were promptly cut so that the heart that stopped in systole could be emptied of blood before it was accurately weighed. This was not possible in the animals found dead (having died during the night). For

TABLE 5.—*Comparative Results*

Group	Number Rabbits	Average Weight, Kg.	Average Weight Loss, Kg.	Pulse Rate	Average Pulse Variation	Death		Data on Heart										Results	
								Weight		Length		Width at Base		Width at Apex		Width at Left Ventricle			
						A	D	A	D	A	D	A	D	A	D	A	D	Positive	Negative
1	10	2.63	0	251	(7) +63 (3) —24	7	3	8.9	12.2	3.4	3.4	2.44	2.76	1.61	2.23	1.8	1.6	8	2
2	11	2.4	0.75	207.3	+82.0	6	5	8.16	11.03	3.83	3.48	2.35	2.56	1.75	1.65	1.66	1.70	10	1
3 A	3	2.72	0.89	242	+40.8	2	1	8.82	6.02	3.35	2.9	2.6	2.5	1.8	1.8	1.7	1.8	3	..
3 B	4	3.2	0.15	224	+36.6	4	0	10.1	0	2.8	0	2.8	0	1.9	0	2.0	0	3	1
4	6	2.16	+0.48	200	0	3	4	8.93	8.84	3.13	2.85	2.46	2.63	1.43	1.83	1.6	1.75	0	6

this reason the weights and measurements of the hearts were divided within the groups. Accordingly, the average weight of the hearts of the rabbits that were killed was the lowest in group 2 (8.16 Gm.), while it was greatest in group 3 B (10.1 Gm.). In proportion to the final body weight, the opposite was true, the hearts of group 2 being $\frac{1}{203}$ of the average body weight as compared with $\frac{1}{301}$ of the average body weight in group 3 B. When one considers the ratio of the average final heart weights to the average final body weights, the largest hearts occurred in the rabbits receiving desiccated thyroid and in those animals operated on in addition to being fed the desiccated thyroid, namely, the rabbits in groups 2 and 3 A, respectively. The average weights of the hearts in group 1 are similar to those in the normal control group. In like manner, the hearts in these two groups (2 and 3 A) were the longest (3.83 and 3.35 cm.). Their transverse diameters were also the narrowest but more uniform considering the measurements from the auriculoventricular grooves to the apexes. Their left ventricular diameters were, however, comparable to those of the controls (group 4). When the hearts of the rabbits in group 1 are compared with those of groups 2 and 3 A, they are found to be somewhat heavier, shorter and wider at the bases although their apexes were slightly narrower. The left ventricular measurements were similar. Although these weights and measurements of the heart were undoubtedly subject to inaccuracies owing to the manner of death, the retention of blood and the limitations of precision, both human and mechanical, there is evidence to support further the conclusion that the augmented heart rate induced hypertrophy after an interval of time and that there eventually resulted disturbance within its architecture. The latter condition may be due to a disproportion between the heart's load of work and its available nutrition.

COMMENT

A comparison of the results shows that lesions were produced in varying degree in the hearts of 80 per cent of the rabbits (group 1) operated on to destroy the inhibitory nerve mechanism, while approximately 90 per cent of the hearts of the rabbits (group 2) made toxic with desiccated thyroid showed similar lesions. It is also evident that a combination of both of these experimental conditions produced more striking results.

The facts that have evolved in this study appear to us to substantiate the claims of other investigators (Goodpasture,[3] Cameron,[4] Hashimoto,[5] Takane [6]) as well as our own that certain lesions do occur in the hearts

3. Goodpasture, E. W.: J. A. M. A. **76**:1545, 1921.
4. Cameron, A. T., and Carmichael, J.: Am. J. Physiol. **58**:1, 1921.
5. Hashimoto, H.: Endocrinology **5**:579, 1921.
6. Takane, K.: Virchows Arch. f. path. Anat. **259**:737, 1925.

of rabbits made toxic by the administration of thyroid products (thyroxine). It also seems to us that such lesions are similar to those described in human hearts by Fahr and Kuhle [7] and Goodpasture,[4] although one might question the conclusion of these authors on the basis of the material used for study. Weller's [8] investigation, in which he was unable to find any lesions in the hearts of thirty-five patients with exophthalmic goiter that could not be matched in the hearts of a control series, is at variance with the studies of these authors. We have not had the opportunity to study adequate and reliable human material, since it appears to us that the evaluation of the effects of hyperthyroidism on the human hearts should be made on the basis of the study of the hearts of persons dying of fulminating hyperthyroidism in the earlier decades of life when the cumulative damages of previous cardiac insults may be excluded or minimized. It is our belief that in older persons with oncoming arteriosclerosis, occupational vicissitudes, etc., the cardiac damage induced by an excess secretion of thyroxine cannot be readily separated from other nonspecific traumatisms of the cardiac muscles.

All clinical observations point to a basic damage of the myocardium in instances of toxic hyperthyroidism although the disease entity is considered as having a more widespread character (Rake and McEachern [9]). Our studies support this point of view.

If one accepts hyperthyroidism as a disease entity in which certain cardiovascular irregularities become dominant clinically and experimentally, there arises the question of the specificity of a toxic influence of an excess thyroxine content of the blood on the cardiac muscle. Yater [10] expressed the belief that it is "increased thyroxin content in the heart muscle which causes the heart to beat more rapidly and more vigorously" and that there is "associated with this a general vascular relaxation brought about by local action of metabolites on the arterioles and capillaries." Rake and McEachern [9] concluded that hyperthyroidism by itself produces no specific lesions in the myocardium. These authors pointed to the absence of glycogen in the experimentally produced disease, as determined by Hoet [11] and de Fauw,[12] as indicating susceptible undernourished muscle that yields readily to damaging influence. Lehrman and Means,[13] in an analysis of one hundred and eighty-four patients

7. Fahr, T., and Kuhle, J.: Virchows Arch. f. path. Anat. **233**:286, 1921.
8. Weller, C. V.; Wanstrom, R. C.; Gordon, H., and Bugher, J. C.: Am. Heart J. **8**:8, 1932.
9. Rake, G., and McEachern, D.: Am. Heart J. **8**:19, 1932.
10. Yater, W. M.: Am. Heart J. **8**:1, 1932.
11. Hoet, J. P., and Marks, H. P.: Proc. Roy. Soc., s. B, **100**:72, 1926.
12. de Fauw, J.: Compt. rend. Soc. de biol. **105**:228, 1930.
13. Lehrman, J., and Means, J. H.: Am. Heart J. **8**:55, 1932.

with hyperthyroidism, concluded that "it is indicated that hyperthyroidism per se does not produce so-called thyroid heart disease but causes functional derangement in a cardiovascular system already damaged by other pathological conditions." From these citations in the literature, it may be seen that the trend of thought of the investigators is first to question the possibility of segregating specific lesions of the heart in hyperthyroidism in clinical or experimental studies and secondly to point out that if such disturbances do occur they are probably nonspecific, resulting from altered conditions in the circulatory system in particular and the body in general.

As stated previously, our own studies lead us to believe that specific lesions can be produced in the hearts of rabbits fed desiccated thyroid within the limits of anticipated individual variations in animals. With regard to the specificity of such lesions, we can only point to the results of our studies in which we attempted to unleash the heart by interference with its stabilizing mechanism. In the rabbits of this series (group 1), it must be remembered, the functional excitation was quite well limited to the heart, there being little or no modification of body weight. It therefore became necessary for such unbalanced hearts to maintain the circulation in otherwise normal rabbits. Under such conditions of rest in cages and with probable individual, variable, cardiac stability and compensation, lesions similar to those occurring in hyperthyroidism appeared in a comparable manner. Although we were not able adequately to demonstrate it microscopically, we strongly suspect that in this group of animals the altered functional strain, the disappearance of glycogen or other nutritional disturbance were responsible for the thyroid heartlike lesions in the rabbits with surgically augmented heart rates.

CONCLUSIONS

Comparable myocardial lesions consisting of swelling of the muscle bundles, loss of cross-striation, fat vacuole, histiocytic invasion and fibrosis with fraying of the muscle bundles were produced in varying degree in 80 per cent of the rabbits in which the depressor nerve mechanism to the heart was destroyed by operation and in 90 per cent of the rabbits with toxicity induced by the administration of desiccated thyroid.

Rabbits operated on to destroy the depressor nerve mechanism to the heart and also given desiccated thyroxine to the point of toxicity developed more striking myocardial lesions.

The administration of doses of ephedrine to raise the pulse pressure did not augment the results, but rather lessened them.

These studies support the view that the myocardial lesions found in hyperthyroidism are nonspecific and probably of mechanical and nutritional origin.

EXPERIMENTAL INTIMAL SCLEROSIS OF THE CORONARY ARTERIES OF RATS

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This paper constitutes a report of some experimental work in which widespread and severe intimal lesions of the coronary arteries were produced in rats by the administration of enormous doses of an agent which exerts a profound effect on the calcium metabolism.

MATERIAL AND METHODS

Fourteen rats, 28 days old, of an average weight of 44 Gm., were used in the experiment. Seven of these were kept in a separate cage and were given, in addition to their regular diet, from 1 to 2 drops per day of activated ergosterol, 10,000 ×. This was given by a dropper by mouth. These rats as well as the seven controls were on our stock laboratory diet. Three of the experimental animals died in the second week of the experiment and were mutilated by the remaining rats so that sections could not be prepared from anything except the bones. One rat died at twenty-one days, and the remaining three were killed on the twenty-second day. The experimental animals lost a slight amount of weight during the experiment, whereas the controls more than doubled their weight.

Autopsy was performed on the three rats which survived for twenty-two days and the one rat which died on the twenty-first day, and tissues were obtained from the heart, aorta, lung, liver, kidney, thyroid gland, parathyroid glands and bones. These were fixed in formaldehyde and sectioned by the paraffin method. Serial sections of the cardiac muscle were cut in each case. They were stained with hematoxylin and eosin.

OBSERVATIONS

Coronary Vessels.—The branches of the coronary arteries in the cardiac muscle showed numerous and severe lesions. They may be interpreted more easily if one is familiar with the lesion produced with one single massive dose of activated ergosterol, 10,000 ×. This has been described in detail (Ham [1]), and the essential finding in it is the tremendous calcification of the media. Cellular structure is often not apparent in this layer, and only a few endothelial lining cells may be seen persisting in the intima.

The medial lesions in the present work are quite obvious, and the dense ring of calcification serves to mark the position of the original

From the Department of Anatomy, University of Toronto.

1. Ham, A. W.: Arch. Path. **14**:613, 1932.

media. This ring of calcification may be advantageously compared to that resulting from a single upset of calcium metabolism (fig. 4 of previous contribution [1]). In contrast to the lesion produced by a single dose of ergosterol, there may be seen in these experimental animals, in the coronary arteries, a marked proliferation of tissue inside the media. This newly formed tissue encroached on the lumen in varying degrees. The new lumen which had formed in each vessel was lined with very cellular endothelium, which gave the impression of recent proliferation. The main bulk of the newly formed tissue was continuous

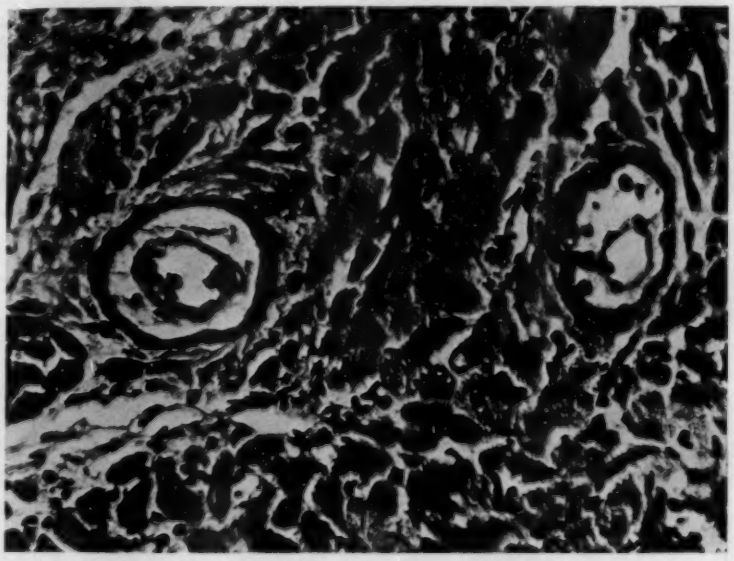

Fig. 1.—Photomicrograph of two coronary vessels showing loosely arranged intimal proliferation. The media of the original vessel wall shows as a heavily calcified ring. Hematoxylin and eosin; × 200.

with, and lay directly beneath, the endothelium. In some instances it consisted of loosely arranged vacuolated connective tissue which possessed an embryonic appearance (figs. 1 and 2). In other instances the tissue was young and cellular, but the matrix was more solid. This type of lesion may be seen in figures 3, 4 and 5. This newly formed connective tissue encroached on the lumen in different degrees (figs. 3, 4 and 5). In many instances it led to an almost complete obliteration of the lumen of the vessel. Some of these vessels in which the lumen had been almost completely obliterated gave the appearance usually associated with recanalization.

Fig. 2.—Photomicrograph of a coronary vessel showing loosely arranged connective tissue resulting from intimal proliferation. The media of the original vessel wall shows as a heavily calcified ring. The cellular proliferation has left two lumens. Hematoxylin and eosin; × 400.

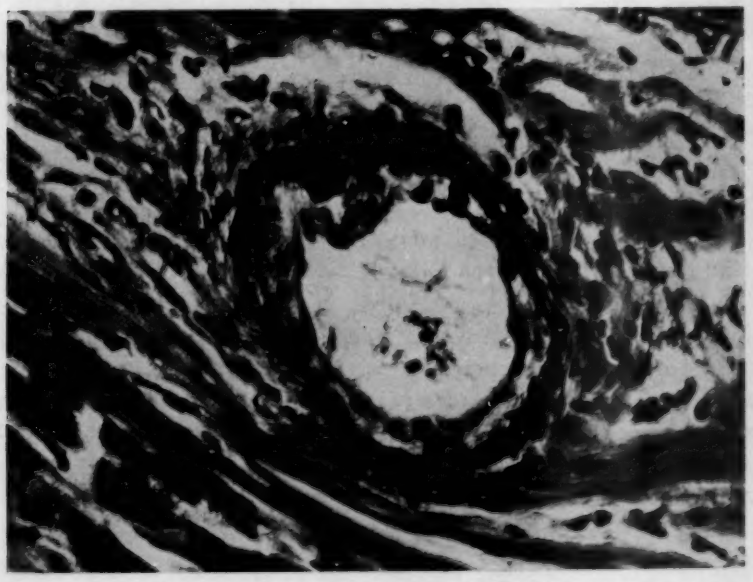

Fig. 3.—Photomicrograph of a coronary vessel showing intimal proliferation of a denser type of tissue. The media of the original wall shows as a heavily calcified ring. Hematoxylin and eosin; × 400.

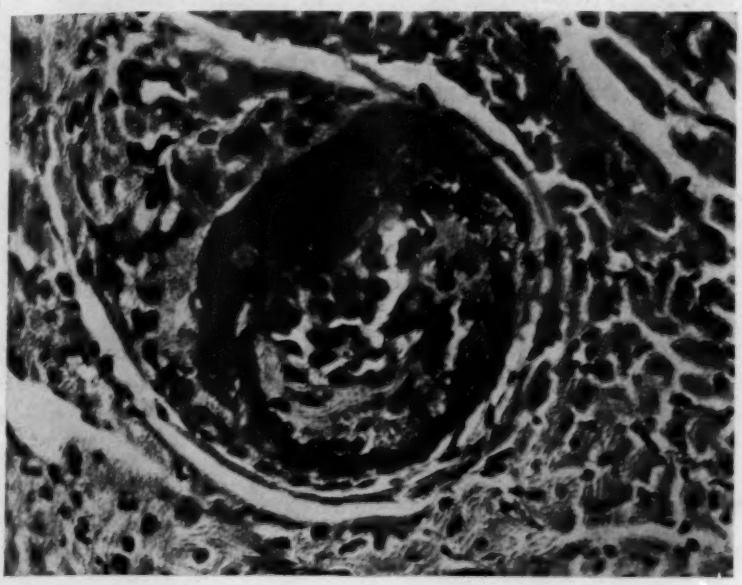

Fig. 4.—Photomicrograph of a coronary vessel in which intimal proliferation has almost occluded the vessel. The media of the original wall appears as a heavily calcified ring. Hematoxylin and eosin; × 400.

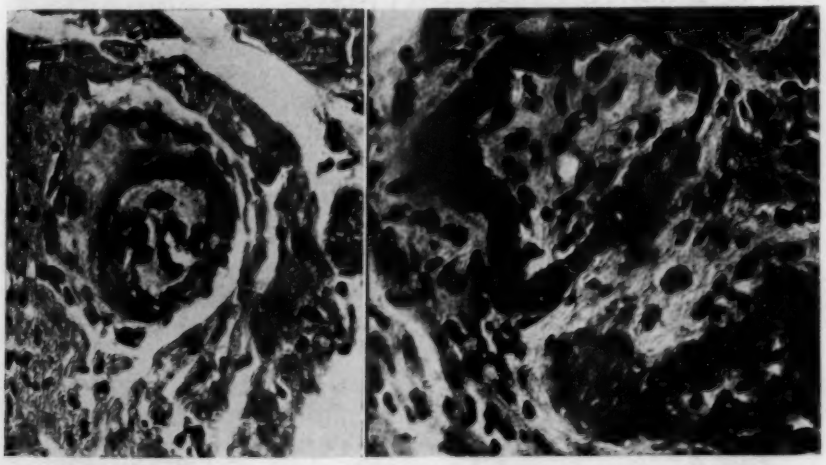

Fig. 5.—Photomicrographs of coronary vessels showing extensive intimal proliferations. The media of the original vessel walls may be seen as heavily calcified rings. Hematoxylin and eosin; left, × 300; right, × 400.

Cardiac Muscle.—Areas of atrophy of the muscle fibers were found in the myocardium, and these were associated with a real or apparent increase of connective tissue and some hemorrhage.

COMMENT

The most important point to discuss in connection with these lesions is their etiology. It is probably misleading to think of them in association with vitamin D because it is improbable that they are produced by the direct effect of this substance on arteries. There is a great deal of evidence to indicate that the medial lesion is produced by a disturbance of mineral metabolism. Vitamin D in special, potent preparations is a convenient agent to use in enormous dosages to cause these disturbances of mineral metabolism. This evidence which points to hypervitaminosis D arteriosclerosis being caused by precipitations of calcium from the serum rather than by preliminary degenerative changes in the arterial tissues may be briefly summarized:

1. The toxicity of vitamin D varies with the amounts of calcium in the animals' diet (Bills and Wirick,[2] Harris and Innes [3]).

2. The same general type of calcification (metastatic) may be produced by overdosages of parathyroid hormone.

3. The medial calcifications occur suddenly following a latent period after the administration of a single massive dose of vitamin D (Ham [1]).

4. The preceding changes in the arterial walls are by no means sufficient to account for the tremendous calcification which occurs a few hours later (Ham [1]).

5. The time of calcification of the vessel wall corresponds with the beginning of the fall of the level of serum calcium after the attainment of a hypercalcemia when one dose of vitamin D is used (Ham and Portuondo [4]).

If the cause of the medial lesion seen after the administration of a single massive dose of activated ergosterol is considered to be the precipitation of calcium from the blood, it becomes necessary to visualize a different sort of sequence of events in this type of arteriosclerosis from that which has been postulated to explain the development of the usual lesion. In hypervitaminosis D arteriosclerosis the deposition of calcium is an early event rather than a late one, and instead of injury being followed by degeneration or proliferation, then calcification, the order is reversed and calcification is followed by degeneration and proliferation.

The intimal proliferation could be explained in several different ways. It might be suggested that it is in the nature of a hyperplasia to compensate for an injured media. It is conceivable that repair might

2. Bills, C. W., and Wirick, A. M.: J. Biol. Chem. **86**:117, 1930.
3. Harris, L. J., and Innes, J. R. M.: Biochem. J. **25**:367, 1931.
4. Ham, A. W., and Portuondo, B. C.: Arch. Path. **16**:1, 1933.

ensue from the intima when the media is so severely damaged by precipitation of calcium. The regeneration of tissue from the intima is, however, sufficiently extensive to suggest that it has been stimulated to grow by more than one shower of calcium. Whereas the effect of the first precipitation seems to be primarily on the media of vessels. it is not improbable that after the media has become well calcified subsequent precipitations would injure the intima to a greater extent than before. Granules of calcified material could be seen in the cellular masses resulting from the intimal proliferation, particularly in the portion of these close to the media.

Whereas it seems probable that precipitations into vessel walls occurred on several occasions during the course of the experiment, it is possible that the deposit of calcium in the wall of the media could also act as an irritant in its immediate neighoborhood. There is some evidence to show that calcium may gradually disappear from arteries (Spies [5]), and this problem is now being investigated in this laboratory. It is possible that the redissolving of the primary deposit might be accompanied by some local disturbances which would stimulate cell growth.

The significance of this work does not appear to us to lie in the fact that the lesions may be caused by hypervitaminosis D. It would appear to us that these intimal lesions, as well as the medial lesions, are the result of nothing more than the inability of the blood to retain, on certain occasions, all of its calcium in solution. The possibility of disturbances of calcium metabolism being a factor in the etiology of human arteriosclerosis should be considered more seriously than it is at present. The blood normally contains more calcium than can be explained by the laws of simple solution. It is possible that there are many ways in which small amounts of calcium could be precipitated from the blood.[6] Whether this mechanism accounts for any lesions in man remains to be seen, but the ease with which endarteritis in the coronary vessels may be produced by this particular method of disturbing calcium metabolism suggests that this etiology of arterial disease (precipitation of calcium) should at least be kept in mind.

CONCLUSIONS

Proliferative changes of a marked character in the intima of the coronary arteries of rats occurred following the daily administration of enormous dosages of activated ergosterol, which profoundly affects the calcium metabolism. It is our opinion that whereas the first effect of the precipitation of calcium from the serum is the calcification of the media, further precipitations or the later effects of the first precipitation result in intimal proliferation.

5. Spies, T. D.: Arch. Int. Med. **50**:443, 1932.
6. Ham.[1] Ham and Portuondo.[4]

HEPATIC AND BILE DUCT CHANGES FROM OBSTRUCTION OF COMMON BILE DUCT DUE TO PANCREATIC CARCINOMA

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Early writers suspected in a general way that long-continued obstruction to the common bile duct might result in increased fibrosis within the liver, although comparatively little attention was paid to this condition in human beings. Albers [1] described the liver in such instances as granular, rough and yellowish brown, with atrophy and induration of the parenchyma, and the probable pathogenesis of these changes was discussed by Virchow.[2] Förster,[3] Cohn [3] and Legg [4] distinguished, as a special type, the cirrhosis arising in connection with biliary obstruction. Charcot and Gombault [5] described similar hepatic lesions resulting from calculi in the ampulla of Vater and in the gallbladder, cancer of the head of the pancreas and angiocholitis of the larger bile ducts. Mangelsdorff,[6] Ruppert,[7] Janowski,[8] Sauerherring [9] and Ford [10] individually emphasized particularly the changes in the bile ducts, the proliferation of the connective tissue or the occurrence of multiple necroses in the

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2. Virchow, R.: Verhandl. d. phys.-med. Gesellsch. **7**:21, 1857.

3. Förster, cited by Cameron, G. R., and Oakley, C. L.: J. Path. & Bact. **35**:769, 1932.

4. Legg, J. W.: St. Barth. Hosp. Rep. **9**:161, 1873; Tr. Path. Soc. London **25**:132, 1874; On the Bile, Jaundice and Bilious Diseases, London, H. K. Lewis & Co., Ltd., 1880.

5. Charcot, J. M., and Gombault, A.: Arch. de physiol. norm. et path. **8**:272, 1876.

6. Mangelsdorff, J.: Deutsches Arch. f. klin. Med. **31**:522, 1882.

7. Ruppert, H.: Zur Frage über die Entstehung der biliären Lebercirrhose, Pam. Towarz. lek. Warszaw **87**:324 and 531; **88**:531, 1891.

8. Janowski, W.: Beitr. z. path. Anat. u. z. allg. Path. **11**:344, 1892.

9. Sauerherring, H.: Virchows Arch. f. path. Anat. **137**:155, 1894.

10. Ford, W. W.: Am. J. M. Sc. **121**:60, 1904.

liver affected by stasis. Eppinger [11] reported a case which may be regarded as classic and in which the common bile duct was inadvertently severed during an operation and immediately ligated 2 cm. distal to the junction of the hepatic and cystic ducts. Icterus and bilirubinemia appeared within thirty-six hours, and death from aspiration pneumonia occurred at the end of forty-eight hours. At autopsy, the question of infection of the biliary tract and of circulatory disturbances was ruled out; microscopically, there were pigmentary and regressive changes, especially in the central cells of the lobules, and dilatation and elongation of the bile capillaries, with rupture of their distal ends into the perivascular lymph spaces. Studies of biliary stasis in man have been contributed by Weber,[12] Abramow and Samoilowicz,[13] Carnot and Harvier,[14] Ogata,[15] Zypkin [16] and Rabl.[17]

Heineke [18] was one of the first to distinguish two distinct types of obstructive biliary cirrhosis in man, the one complicated by infection and the other uncomplicated. More recently Fiessinger, Albot and Dieryck [19] referred to the hepatic lesions resulting from pure mechanical stasis as cholestatic cirrhosis in contrast to the infectious type or cholangeitic cirrhosis. MacMahon and Mallory [20] described the characteristics of noninfectious obstructive cirrhosis; their findings, however, are open to the possible criticism that infection may have been a complicating factor in the cases in which there were calculi and acquired strictures of the common duct and perhaps also in some of those in which carcinoma of the ampulla of Vater was present.

In animals, aseptic ligation of the common bile duct is a simple procedure, as a result of which there is a vast literature on the subject, but one of the few diseases in man which appropriately reduplicates this experimental lesion and adequately fulfils the requirements of the condition of uncomplicated biliary stasis is obstruction to the common bile duct by carcinoma of the head of the pancreas. This condition is comparatively uncommon, and before death there is usually only a relatively short period of stasis, the effects of which are further mitigated to a

11. Eppinger, H.: Beitr. z. path. Anat. u. z. allg. Path. **31**:230, 1902.
12. Weber, F. P.: Tr. Path. Soc. London **54**:103, 1903.
13. Abramow, S., and Samoilowicz, A.: Virchows Arch. f. path. Anat. **176**: 199, 1904.
14. Carnot, P., and Harvier, P.: Arch. de méd. expér. et d'anat. path. **19**:76, 1907.
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16. Zypkin, S. M.: Virchows Arch. f. path. Anat. **262**:791, 1926.
17. Rabl, R.: Beitr. z. path. Anat. u. z. allg. Path. **86**:135, 1931.
18. Heineke, H.: Beitr. z. path. Anat. u. z. allg. Path. **22**:259, 1897.
19. Fiessinger, N.; Albot, G., and Dieryck, J.: Ann. d'anat. path. **8**:537, 1931.
20. MacMahon, H. E., and Mallory, F. B.: Am. J. Path. **5**:645, 1929.

variable extent by the functional activity of the gallbladder, which is usually normal. Judd, Counseller and McIndoe [21] have emphasized the development of hydrohepatosis in association with obstruction to the common bile duct by pancreatic carcinoma, in which condition they felt that, owing, among other things, to the absence of infection, there is a minimum of obstructive biliary cirrhosis. Rolleston [22] has maintained that in man cirrhosis does not result from uncomplicated biliary obstruction per se. The paucity of knowledge of the hepatic lesions produced in the condition of uncomplicated biliary stasis was recently emphasized by Rössle,[23] who pertinently stated: "Besieht man kritisch die Literatur, so schwindet die ungeheure Masse der angeblich hierher gehörigen Beobachtungen auf ein Häufchen zusammen." (If one reviews the literature critically, the enormous mass of observations that apparently belong in this group shrinks to minute proportions.)

The purpose of the present communication is to describe the changes in the hepatic and bile ducts resulting from obstruction to the common bile duct by carcinoma of the head of the pancreas, and to submit evidence showing that cirrhosis may result from uncomplicated or noninfectious biliary stasis. The material for this study was obtained from twenty-four cases of primary carcinoma of the head of the pancreas with obstruction to the common bile duct. Seven of these cases are from the pathologic laboratory of the Pennsylvania Hospital; Dr. John T. Bauer placed this material at our disposal. None of the cases was complicated by previous cholecystitis or cholelithiasis, terminal abscesses or operative decompression of the biliary system, and in all of them comprehensive records and suitable gross or histologic material or both were available for use. Histologic material was fixed in formaldehyde or Zenker's fluid; some of it was frozen and sectioned, and the remainder was blocked in paraffin, cut and stained with phosphotungstic acid, hematoxylin-eosin, scarlet red, methylthionine chloride, U. S. P. (methylene blue), van Gieson's and Mallory's connective tissue stains, Verhoeff's elastic tissue stain, oxidase stain, iron-alum-hematoxylin stain, Wilder's modification of the silver diaminohydroxide stain, Mallory's potassium ferrocyanide stain and McIndoe's adaptation of the Hortega silver carbonate stain for bile canaliculi. In many instances complete sets of serial sections were also examined.

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21. (*a*) Judd, E. S., and Counseller, V. S.: J. A. M. A. **89**:1751, 1927. (*b*) Judd, E. S., and McIndoe, A. H.: Collected Papers of the Mayo Clinic and Mayo Foundation, Philadelphia, W. B. Saunders Company, 1929, vol. 21, p. 158. (*c*) Counseller, V. S.: Ann. Surg. **87**:210, 1928.

22. Rolleston, H.: Diseases of the Gallbladder and Bile-Ducts, in Christian, H. A., and Mackenzie, James: Oxford Medicine, New York, Oxford University Press, 1932, vol. 3, pp. 390 and 469.

23. Rössle, R., cited by de Josselin de Jong, R.: Compt. rend. de conf. internat. de path. geogr., 1931, p. 38.

Metastatic nodules were present in the liver in many of the cases included in this study. The changes in the hepatic tissue adjacent to metastases are well known, and since they have no bearing on the changes under consideration, they will not be discussed further.

GROSS CHANGES

The common bile duct averaged 3 cm. in diameter, the dilatation usually being uniform, although sacculations occasionally occurred; the right hepatic duct was less distended than the left, and the intrahepatic ducts suffered least. The walls of the larger biliary passages first become attenuated and subsequently undergo slight thickening. In the early stages the contents of the bile passages are not much altered but later they become thick and tenacious and contain precipitated granules of inspissated pigment. In two cases, the contents of the gallbladder and ducts consisted of thin, pale, yellowish-green fluid. The liver showed a mixture of yellow, olive-drab and deep green mottling, and was usually enlarged and frequently smooth, although in four cases it was smaller than normal and in nine cases the surface was either finely or coarsely granular. Weights of from 1,800 to 2,000 Gm. were recorded in cases in which metastatic nodules were few or absent. In two cases the liver weighed 810 Gm. and 840 Gm., respectively. Varicose ducts may appear on the surface, and the organ may feel cystic. In the cases lasting a long time, the liver was firm, tough and heavy, cut with increased resistance and on section disclosed enormously dilated biliary passages and visible small islands of connective tissue which in a few instances was a well marked feature. The intrahepatic bile passages were usually filled with deep green or greenish-black, thick, viscid bile, which in two cases was pale yellow. The lobular markings were accentuated by contrast with the darkly colored centers and light peripheries.

MICROSCOPIC EXAMINATION

Bile pigmentation is a variable feature although its intensity is always greatest about the central vein and branches of the hepatic vein. Within hepatic cell cytoplasm bile pigment occurs in three forms: (1) as a diffuse yellowish-green staining substance without precipitation, (2) as fine, golden brown granules and (3) as variously sized, yellowish-green or greenish-black droplets. It may be scattered diffusely throughout the cell, or may be clumped as small foci at one or both poles of the nucleus, which it may completely encircle or occasionally overlie and obscure. Rarely, the nucleus itself seems to have taken up some of the pigment. In the intracellular, cytoplasmic vacuoles, the outer circumference may show a concentration of the pigment, which may also be contained as fine granules within the vacuole itself. Occasionally a

narrow zone of the hepatic cell cytoplasm bordering the canaliculus is deeply pigmented. Bile thrombi or bile cylinders are constantly present, and may be rod-shaped or cylindric, occasionally branched and sometimes markedly irregular owing to their projection into collateral canaliculi. In the necrotic areas bile thrombi are discharged from the canaliculi into the tissue spaces and in the later stages into the sinusoids; in both situations they may be found lying free or phagocytosed in part or in whole. Bile masses also form casts in a number of the smaller interlobular ducts which on rupture permit extrusion of their contents into the adjacent stroma or into a dilated lymphatic. Monocytes laden with bile pigment are found within the lobule, in the stroma of Glisson's capsule and wherever else a reactive or reparative process is in progress. In the peripheral and central portions of the lobules of certain of the livers iron pigment was present in the hepatic and Kupffer cells, and what appeared to be lipochromes were present in the hepatic cells.

About the central vein and branches of the hepatic vein, the hepatic cells show regressive changes which vary in intensity from simple parenchymatous degeneration to actual necrosis and ultimate dissolution. Approaching the periphery of the lobule, the cells retain more of their normal appearance and regular cordlike arrangement, although less marked sporadic regressive changes may be present. Occasionally a narrow band showing pigmentation and regressive changes may radiate from the central vein to the portal radicle, where it is not uncommonly joined by a similar band from an adjacent lobule. Atrophy of the hepatic cells is noted in association with dilated sinusoids and is present especially at the periphery of the lobules, where irregularly distributed intracellular cytoplasmic vacuoles may be found.

Uniform distention of bile capillaries is a constant early feature, and, with subsequent disintegration of the polygonal cells, on the integrity of which the continuity of the canalicular system depends, rupture takes place. The dilating collateral canaliculi not infrequently extend to the perivascular tissue space, exerting pressure against and indenting the reticular wall, which finally gives way at this point with resulting extrusion of the canalicular contents into the sinusoid. Pictures suggesting the existence of intracellular canaliculi are not observed. There is no blood present within canalicular lumens.

The arteries and arterioles show an increase in the medial musculature and less frequently subintimal proliferation which may result in partial or complete obliteration of the lumens. The branches of the portal vein are regularly dilated, and their walls are thickened by a perivascular fibrosis; their lumens are occasionally occluded by mottled or by tumor thrombi. The lymphatic vessels in Glisson's capsule are dilated and at times contain bile pigment. The central vein is usually dilated, and its wall is variably thickened by hyalinized connective tissue.

Branches of the hepatic vein are dilated and congested and may contain a few lymphocytes and single or multinucleated macrophages laden with bile pigment; their walls are considerably thickened by hyalinized connective tissue. Sinusoidal hyperemia is encountered regularly about necrotic areas, occurs indifferently in areas of atrophy of the hepatic cells and is a variable feature, depending in many instances on extraneous factors. In cases of long duration there is an appreciable increase of reticulum in the walls of the sinusoids, the lumens of which may contain swollen, pigmented or nonpigmented Kupffer cells and monocytes, an occasional lymphocyte and polymorphonuclear leukocyte, iron pigment and free bile pigment in the form of droplets, coarse granules and bile masses. Rupture of the sinusoidal walls, permitting a direct communication with tissue spaces and with bile canaliculi, is a late feature.

In areas where bile pigmentation is marked or regressive changes are in progress, the Kupffer cells are prominent and contain bile pigment, nuclear fragments and other débris. In necrotic areas these cells also show regressive changes but remain remarkably well preserved long after complete dissolution of the polygonal cells has occurred. When disintegration sets in, the nucleus disappears before the cytoplasm breaks up.

Monocytes are constantly found in the interlobular connective tissue, surrounding and invading areas of necrosis, within sinusoids and other blood vessels and occupying the perivascular tissue spaces. Together with the Kupffer cells, from which they are at times difficult to distinguish, they manifest marked phagocytic properties and occasionally form multinucleated giant cells.

Aside from the regressive changes occurring about the central veins, there were two other distinctive types of necrosis:

1. In sixteen of the cases, small, isolated, rectangular or oval, sharply demarcated, focal necrotic areas, consisting at first of from 8 to 10 polygonal cells, were found in the midzones of many of the lobules. The regressive changes in these areas were characterized by: (*a*) fading and shrinking of the nuclei and fading of the cytoplasm of the hepatic cells with accentuation of the peripheral cellular outline due partly to swelling and concentration of the reticulum, (*b*) a relatively greater resistance of the Kupffer cells toward retrogressive changes, (*c*) a moderate infiltration of monocytes and (*d*) a sharply defined peripheral limit, with normal cells on the outside and necrotic cells within. The amount of pigment contained in these areas was not greater than that present in the adjacent unaffected cells, and gradually diffused out to the periphery where it was engulfed by inwandering phagocytic cells. This lesion may enlarge by projecting its apex to and sometimes beyond the central vein,

and more frequently by extending its broad base into the outer third of the lobule, reaching at times to the portal radicle, where it may be met by a similar lesion (fig. 1). The area was usually bloodless but was not infrequently surrounded by a hyperemic and hemorrhagic zone, into which the necrosis might extend secondarily. A mantle of monocytes and lymphocytes was noted about those recently formed. Within the lesion the normal hepatic architecture was in most instances maintained,

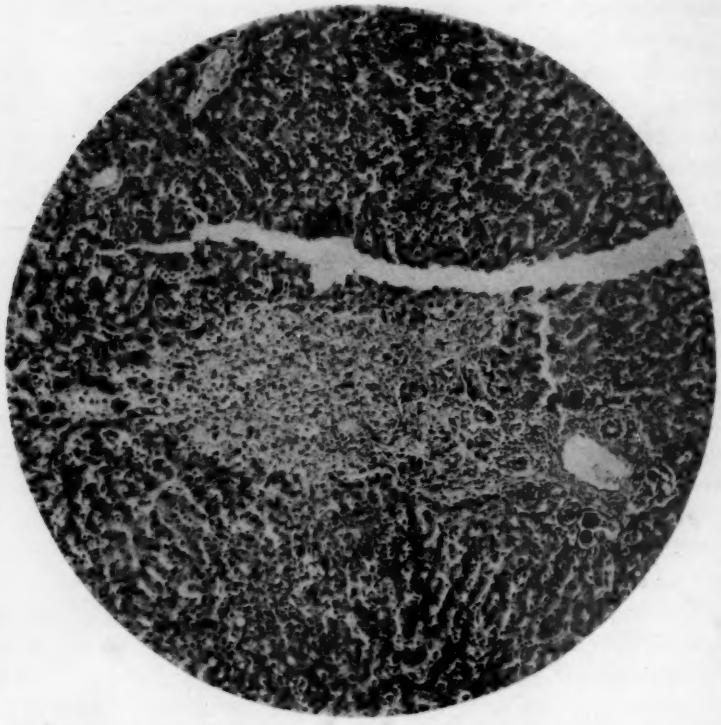

Fig. 1.—An enlarged focal midzonal area of necrosis with its apex at the central vein on the left and its base at the portal radicle on the right. Note also the independent hepatic cell pigmentation about the central vein; × 69.

but finally, with complete dissolution of the cells, nothing remained but a loose meshwork of condensed, thickened, hyalinized, reticular fibers with little or no attempt at organization.

2. In five cases large, oval or triangular, sharply demarcated biliary necroses were present. These occupied, as a rule, the outer portions of the lobules, although some were centered in the portal radicles to which they were entirely confined or from which they extended to involve the peripheries of adjacent lobules. When first formed, the center of

this lesion is composed of a dense, compact, sharply circumscribed, dark green mass of bile pigment, occasionally containing a few scattered ductal epithelial cells. This mass, occupying an intralobular situation, becomes surrounded by a narrow concentric zone of degenerated and necrotic hepatic cells which maintain for some time their normal cord-like arrangement, separated by congested sinusoids lined by relatively unchanged Kupffer cells (fig. 2). Beyond this zone the sinusoids are

Fig. 2.—Intralobular biliary necrosis is present in the center, with a portal radicle below and to the right. Note the disintegration of the central masses of bile pigment, which are separated by coarse reticular fibers impregnated with bile pigment. The surrounding necrotic zone, which is sharply demarcated from the normal hepatic tissue, contains swollen Kupffer cells and infiltrated polyblasts; × 82.

markedly congested and filled with normal or conglutinated erythrocytes. As the lesion progresses the necrotic zone becomes paler and wider, and a fine hepatic cell cytoplasmic vacuolation appears, followed by karyorrhexis and other nuclear changes. The peripheral portion of the central mass of pigment gradually diffuses into the necrotic zones. Coincident with this monocytes wander in and form irregularly distorted, multi-

nucleated giant cells, and the pigment is found in smaller homogeneous and coarsely granular, discrete aggregations. A little later, in the center of the biliary necrosis a swollen, reticular, beaded framework, impregnated diffusely or with coarse granules of bile pigment, becomes apparent, and in the interstices of their fibers many swollen monocytes may be noted. Ultimately, the central mass completely disappears, leaving only a coarse golden yellow reticulum. The lesion becomes more

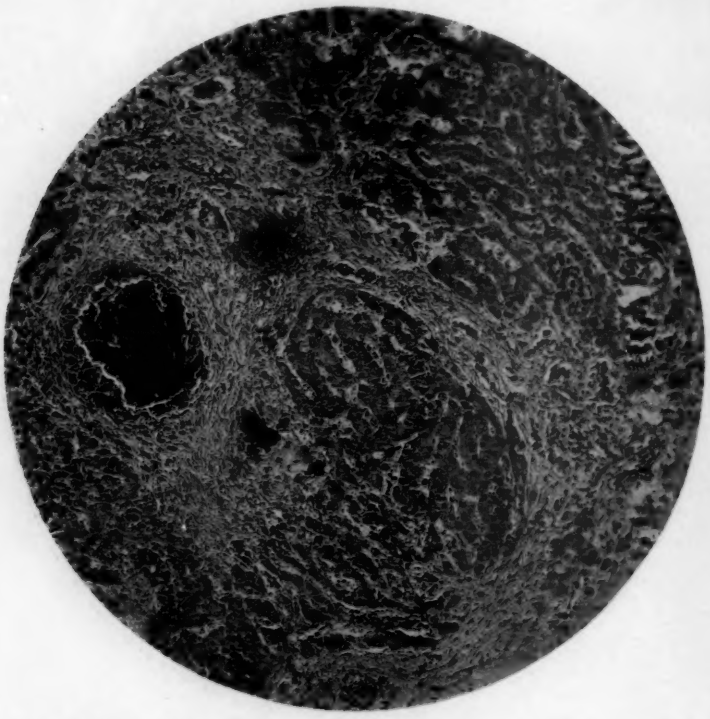

Fig. 3.—Organizing biliary necrosis contributing to perilobular fibrosis. The necrosis is confined to the portal radicle, and shows little diffusion of the central mass of bile pigment; × 103.

or less completely surrounded by large swollen monocytes and lymphocytes accompanied by a few fibroblasts, which may proliferate rather rapidly to form a complete, sharply delimiting wall. Finally the entire outer zone is replaced by more or less dense hyalinized connective tissue which, following its usual course of slow contraction, encroaches on the central contents, rendering them more compact. The biliary necroses occurring within the portal areas show little diffusion of the central masses of bile pigment and only slight retrogressive changes in the surrounding connective tissue, which quickly forms a dense capsule about the lesions, which may persist in this form indefinitely (fig. 3).

The bile ducts become elongated, stretched and tortuous, and in addition show a numerical increase, as evidenced by budding and branching and by proliferation of the lining epithelial cells with the formation of syncytial masses (fig. 4). Regressive changes, consisting of pigmentation, degeneration, atrophy, necrosis and desquamation of individual or isolated groups of cells and rarely of denudation en masse of the lining of the duct leaving a bare basement membrane, are incon-

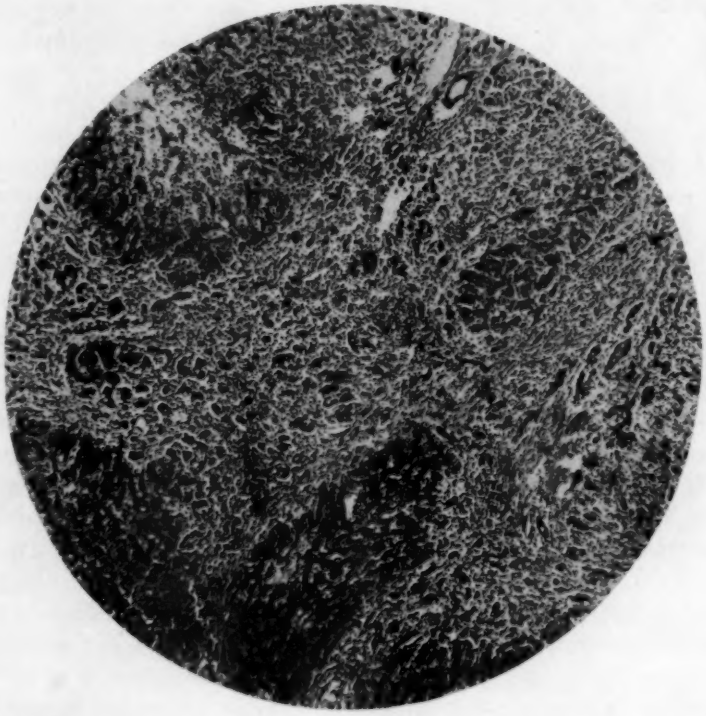

Fig. 4.—Extensive proliferation of the bile ducts and connective tissue; × 60.

spicuous features. The lumens may be empty or filled with bile thrombi, or with a pale, homogeneous, pink-staining material. The walls of the larger ducts are thickened, their lumens are dilated, and the epithelial lining cells usually appear fairly normal.

The portal areas, appearing frequently as large oval structures, show an increase in connective tissue which, in the early stages, closely parallels the changes in the bile ducts but which subsequently develops independently, insinuating itself for varying distances between adjacent lobules, where it joins prolongations from contiguous portal areas. These branching processes invade the outer and, at times, the middle third of the lobule. The individual or small groups of hepatic cells thus

enmeshed gradually atrophy and finally tend to undergo complete disintegration. As a result, the lobule becomes irregular in outline and reduced in size. With total stasis existing for more than two months, proliferation of the connective tissue becomes a striking feature, forming regularly an incomplete, and at times a complete, perilobular collar of fibrosis (figs. 3 and 4). Well developed bands of fibrous tissue surrounding the majority of the lobules were present in only one of our cases. In several others the process stopped just short of this. The subcapsular proliferation of connective tissue was marked in the majority of the cases. The organization of the biliary necroses contributed in certain instances to the perilobular increase of connective tissue (fig. 3). Monocytes, lymphocytes and an occasional granulocyte, as well as masses and granules of bile pigment, free and in phagocytic cells, were present within the newly formed connective tissue, which consisted essentially of young fibroblasts with an abundance of coarse, edematous, fibrillar material, directly continuous with the increased reticulum of the periphery of the lobule.

COMMENT

Legg,[4] Weber[12] and later McMaster, Broun and Rous[24] emphasized the analogy between hydronephrosis and biliary stasis (hydrohepatosis). In both, as in conditions of obstruction to the ducts of secreting glands in general, secretory stasis, dilatation of ducts, vascular disturbances from collateral pressure and parenchymal atrophy occur. In the case of the liver, however, the activities of the gallbladder partially alleviate the effects of stasis. After total obstruction, the hepatic cells continue to excrete until the pressure in the conducting system rises to a certain point (350 mm. of bile in the dog), when the product of their activity is returned to the organism. Pyroxylin (celloidin) casts of the biliary and vascular channels in the condition of obstruction of the common duct by carcinoma of the head of the pancreas demonstrate a tremendous volumetric increase of these channels (Judd, Counseller and McIndoe).[21] Judd, Counseller and McIndoe believed that the intimate relationship between the biliary and vascular passages creates an ideal situation for the production of venous strangulation with consequent diversion of the portal blood current and anemia of the hepatic tissue. The parietal sacculi or diverticuli are effaced, with the result that the walls become smooth. The vasa aberrantia either are flattened out along the walls of the ducts or become enlarged, sacculated and indistinguishable from bile ducts.

In two of our cases, the ductal contents were thin and pale yellow, but "white bile" was not encountered, probably because the obstructed

24. McMaster, P. D.; Broun, G. O., and Rous, P.: J. Exper. Med. **37**:685, 1923.

biliary passages were not the seat of previous disease. In cases of long-standing obstruction, the wall of the gallbladder hypertrophies and undergoes some fibrous thickening, which is probably due, as in the walls of the ducts, to the continuous absorption of bile through the lymphatics. This would tend ultimately to abolish the functions of the gallbladder and predispose to the formation of white bile.

To account for the deeper pigmentation of the center of the hepatic lobule, Legg [4] reasoned that the cells nearest the bile ducts could more easily discharge their contents. Bürker,[25] however, believed that the hepatic lobule consists of a central secreting and a peripheral absorbing portion. As for the bile thrombi, Wyss [26] and Legg regarded them merely as casts of the bile capillaries, but subsequent investigators considered them as an expression of toxic damage to hepatic cells and to be composed of coagulated, inspissated, bile-stained protein (Eppinger,[4] Ogata [15] and Lepehne [27]) or a mixture of lipoid and protein (Rous [28]). Kretz [29] believed that the bile thrombi result from destruction of erythrocytes by the action of bile acids with subsequent iron pigmentation of the hepatic cells. Aschoff [30] emphasized Kodama's [31] finding that in obstructive icterus iron liberated from broken-down hemoglobin is retained in the Kupffer cells and appears only in very small quantities in the polygonal cells of the liver. The occurrence of iron pigment in our cases seems to be of no particular significance either as a criterion or as a contributing factor in the production of the hepatic lesions. Hepatic lipochromes, which Connor [32] demonstrated microscopically and by chemical analysis, have been regarded by Ogata as autochthonously associated with the lipoids present in the liver, which, however, contains no neutral fat.

The vacuoles which occur indiscriminately within the hepatic cells have been considered as mucinous (Chambard [33]) or fat droplets (Canalis,[34] Lehousse [35] and Gerhardt [36]) or as representing a stage

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26. Wyss, O.: Virchows Arch. f. path. Anat. **35**:533, 1866.

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33. Chambard, E.: Arch. de physiol. norm. et path. **4**:718, 1877.

34. Canalis, P.: Internat. Monatschr. f. Anat. u. Physiol. **3**:216, 1886.

35. Lehousse, E.: Arch. de biol. **7**:187, 1887.

36. Gerhardt, D.: Arch. f. exper. Path. u. Pharmakol. **30**:1, 1892; Verhandl. d. Cong. f. innere Med. **15**:460, 1897; München. med. Wchnschr. **52**:889, 1905.

of necrosis resulting from circulatory disturbances (Tischner [37]). We agree with Ogata that they are not due to infiltration of fat or glycogen and probably represent a phase of hydropic degeneration.

In his case Eppinger noted dilatation of the bile capillaries and elongation of their distal ends with rupture into the lymph spaces. This finding is most unusual, for in our cases in which obstructive jaundice was present from seventy-two hours onward we were unable to observe ruptures except in those lasting a longer period. A number of investigators (Harley,[38] Frey,[39] Jagic,[40] Abramow,[41] Abramow and Samoilowicz,[13] Tischner [37] and Naunyn [42]) advanced the view that rupture of bile capillaries occurs directly into the blood stream. Liebermeister [43] felt that with increasing pressure in the canaliculi the bile may diffuse, without rupture, directly into the lymph or blood stream, or, as stated by Minkowski,[44] bile pigments may theoretically easily pass by the process of parapedesis from the damaged hepatic cell directly into the blood stream or perilymphatic space instead of into the lumen of the canaliculi. Sterling [45] could not find ruptured canaliculi until comparatively late in the condition, and Ogata [15] was unable to demonstrate any time relationship between the appearance of icterus and visible canalicular ruptures. Studying the livers of dogs with obstruction to the common bile duct, Barron and Bumstead [46] suggested that bile merely diffuses from the canaliculi into the perivascular tissue spaces, since only dilatation of the canaliculi with formation of bile thrombi could be demonstrated within from six to eleven hours (Barron and Bumstead,[46] Hiyeda [47] and Kodama [31]), and ruptures were not evident within three days (Bloom [48]). Rabl [17] and Scheunert [49] concluded that in human beings and in dogs obstructive icterus does not depend on rupture of the bile capillaries. Haberland [50] and Garnier and Prieur [51] maintained that the classic concept of the mechanical origin of biliary

37. Tischner, R.: Virchows Arch. f. path. Anat. **175**:90, 1904.
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50. Haberland, H. F. O.: Arch. f. klin. Chir. **130**:248, 1924.
51. Garnier, M., and Prieur, R.: Presse méd. **38**:1305, 1931.

retention in lesions of the common duct is incorrect, and that obstructive jaundice really represents a type of toxic or infectious jaundice. They believed that experimental ligation of the common bile duct brings about only transitory hyperbilirubinemia, incapable in itself of producing jaundice, which, when it develops later, they considered as always due to a superimposed toxic or infectious factor. Carrié [52] disagreed with this view, and Chabrol, Maximin and Busson,[53] while admitting that a secondary toxic factor may influence the production of jaundice in obstruction of the common duct, showed that in the beginning, at least, icterus is due to the mechanical factor of stasis. Following the induction of biliary obstruction, both the blood sinusoids and the lymph capillaries enter intimately into the mechanism of production of hyperbilirubinemia, the former being considered the most important by Whipple and Hooper,[54] whereas Barron and Bumstead [46] favor the latter. It is probable that in obstructive icterus the contents of the biliary conducting system are constantly being depleted by absorption and renewed by excretion from the polygonal cells, although not all of the bile entering the hepatic cells is necessarily excreted into the canaliculus, but may pass instead directly back into the perivascular tissue space, the blood stream or both.

Despite the fact that each of the three forms of necrosis occurring in the liver affected by stasis presents distinct and easily recognizable morphologic characteristics, no consistent attempt has been made to differentiate between them on the basis of pathogenesis and etiology. Charcot and Gombault [5] suggested that with increasing pressure in the canaliculi, rupture with extrusion of bile into the parenchyma results in destruction of hepatic cells, a view supported by Harley,[38] Frey,[39] Eppinger,[11] Abramow and Samoilowicz,[13] Carnot and Harvier [14] and Richardson.[55] Foà and Salvioli,[56] Pick [57] and Tsunoda,[58] finding no canalicular ruptures, considered the responsible factor to be the increasing pressure resulting from bile stasis. Gerhardt,[36] Richardson,[55] Ogata,[15] Rous and Larimore,[59] Hiyeda,[47] Rabl [17] and Kikuchi,[60] although recognizing the presence of canalicular ruptures, believed this to be of little importance in comparison with the chemical effects of stagnant

52. Carrié, P. A.: Bull. méd., Paris **45**:913, 1931.
53. Chabrol, E.; Maximin, M., and Busson, A.: Presse méd. **39**:892, 1931.
54. Whipple, G. H., and Hooper, C. W.: Am. J. Physiol. **42**:544, 1917.
55. Richardson, M. L.: J. Exper. Med. **14**:401, 1911.
56. Foà, P., and Salvioli, G.: Arch. per le sc. med. **2**:1, 1877.
57. Pick, E.: Ztschr. f. Heilk. **11**:117, 1890.
58. Tsunoda, T.: Virchows Arch. f. path. Anat. **193**:213, 1908.
59. Rous, P., and Larimore, L. D.: J. Exper. Med. **31**:609, 1920; **32**:249, 1920.
60. Kikuchi, S.: J. Orient. Med. **16**:12, 1932.

bile. Géraudel [61] looked on the lesions as representing a postmortem change, an idea refuted by Carnot and Harvier; [14] likewise, Jagic's [40] view that they arise from progressive inflammation was disproved. Vascular disturbances resulting from arterial thickening (Chambard [33]) or from portal constriction by dilating bile ducts (Tischner,[37] Judd and McIndoe,[21b] MacMahon, Lawrence and Maddock [62]), combined with the effects of toxic bile diffused (Janowski [8] and Loeffler [63]) or extruded from ruptures (Beloussow,[64] Ruppert [7] and Steinhaus [65]) in the canalicular system, are other factors which have been advanced to explain the causation of the necroses the development of which has been thought to be further enhanced by lack of nutrition and impaired regenerative ability of the remaining hepatic cells (MacMahon and Mallory [20]).

In our preparations focal midzonal necroses appeared at any time following obstruction of the common bile duct, being most common in the first two months, and having also been found in the sections of the liver of a patient dying three days after the appearance of jaundice. Vascular changes in the form of hypertrophy of the walls of the arteries and arterioles, thickening of the walls of the hepatic and central veins and sinusoids and tumor thrombosis of branches of the portal vein were observed. However, the sclerotic vascular changes are comparatively late developments in the liver affected by stasis and therefore cannot be held to account for the early appearing necroses, which are also frequently absent in many areas where portal vein and sinusoidal tumor thrombosis is a well marked feature. Vascular constriction by dilating bile ducts was not a particularly outstanding feature in our preparations, which, of course, were not especially designed to demonstrate this change. Moreover, the human body possesses remarkably finely adjusted compensatory mechanisms for overcoming resistance to the flow of blood (McNee [66]) although the structure and function of the circulatory system may vary somewhat in other animal groups (Cameron and Mayes [67]). The nature and efficiency of the collateral circulation in the dog and cat, and especially in man, are of the utmost importance. Numerous anastomoses exist, on the one hand, between the capillary plexuses of the hepatic arterial branches and those of the vessels entering the liver by way of the common bile duct and portal vein, and, on the other hand, between the terminations of the portal vein

61. Géraudel, E.: J. de physiol. et de path. gén. **8**:69, 1906.

62. MacMahon, H. E.; Lawrence, J. S., and Maddock, S. J.: Am. J. Path. **5**:631, 1929.

63. Loeffler, L.: Virchows Arch. f. path. Anat. **265**:41, 1927.

64. Beloussow, P. N.: Arch. f. exper. Path. u. Pharmakol. **14**:200, 1881.

65. Steinhaus, J.: Arch. f. exper. Path. u. Pharmakol. **28**:432, 1891.

66. McNee, J. W.: Brit. M. J. **1**:1017, 1068 and 1111, 1932.

67. Cameron, G. R., and Mayes, B. T.: J. Path. & Bact. **33**:799, 1930.

and hepatic artery. Conversely, a collateral circulation limited in its development and responding slowly to the stimulus of a deficiency in oxygen affords little help under the conditions of biliary stasis. Solowieff [68] and, more recently, Rous and Larimore [59] pointed out that occlusion of branches of the portal vein is merely attended by a progressive parenchymal atrophy.

The livers of rabbits and guinea-pigs excrete relatively large amounts of bile at comparatively low pressures, and at the beginning of stasis develop necroses which, however, are said to not appear in the livers of dogs in which animal the amount of bile secreted is small but the average pressure is high; the total quantity of bile produced is perhaps of more importance than the pressure at which it is secreted. Some investigators have claimed that human bile is innocuous and incapable of causing permanent hepatic damage, but if this is true, as Rous and Larimore [59] have pointed out, man differs from all other well studied animals in which, without exception, aseptic bile results in important local changes. The early observation that the degree of hepatic pigmentation is not paralleled by the development of necrosis is supported by the results obtained by Horrall,[69] Greene and Snell,[70] Horrall and Carlson [71] and Still,[72] who demonstrated bilirubin to be an inert substance in contrast to the bile acids, the toxic properties of which reside in the cholate radicle. By injecting fresh bile into the livers of rabbits and dogs, Hiyeda [47] produced areas of necrosis; Cameron and Oakley,[73] however, were unable to duplicate these observations in the rat even after ligation of the common bile duct had been performed with the idea of minimizing absorption of bile by the smaller ducts. If the focal midzonal areas of necrosis in the human being are due to the effects of extravasated bile, the quantity must be exceedingly small, for they show no pigmentation in excess of that found in adjacent hepatic cells. Furthermore, there are no transitional stages between the focal midzonal necroses and the biliary necroses which represent a true reaction of the tissue to extravasated bile. It is conceivable that coincident with early circulatory changes and increased intraductal pressure in the liver there is a lowering of oxygen tension resulting in nutritional disturbances of hepatic cells and in the production of intermediate metabolic products possibly of the nature of toxic amines. Furthermore, since the liver lies directly in the pathway of the portal blood carrying products absorbed from the

68. Solowieff: Virchows Arch. f. path. Anat. **62**:195, 1875.
69. Horrall, O. H.: J. Lab. & Clin. Med. **14**:217, 1928.
70. Greene, C. H., and Snell, A. M.: J. Biol. Chem. **78**:691, 1928.
71. Horrall, O. H., and Carlson, A. J.: Am. J. Physiol. **85**:591, 1928.
72. Still, E. U.: Am. J. Physiol. **88**:729, 1929.
73. Cameron, G. R., and Oakley, C. L.: J. Path. & Bact. **35**:769, 1932.

intestinal tract, the necrotic hepatic lesions may represent the effects of split protein products resulting from intestinal putrefaction due to the absence of bile.

The biliary necroses probably largely represent, as MacMahon and Mallory [20] have emphasized, the cellular reaction to inspissated bile, which, as a result of pressure, has escaped in the form of tubular masses from attenuated and ruptured ducts, remnants of which can often be recognized. These bile masses act as foreign bodies and are soon attacked by monocytes and foreign body giant cells, while still later fibroblasts proliferate about them and traverse their substance, giving them the appearance of old, partially organized thrombi. The bile mass undoubtedly contains toxic substances which are responsible for the enveloping necrotic zones which are always quite conspicuous when any part of the lobule is involved. Some of these lesions are probably also due to rupture of the canals of Hering and of the collecting tubules of Letulle, for the former are notoriously fragile even in normal preparations. This view is supported by the observation that fluid introduced under pressure into the common bile duct becomes extruded into the hepatic tissue in immediate relationship to the canals of Hering (Chrzonsczczewsky,[74] Heidenhain,[75] Stern,[76] Beloussow [64] and Pfeiffer [77]) or more centrally within the lobule (Bürker [25]). Cameron and Oakley's [73] reconstructions in the rat demonstrate a constant close relationship between hepatic necroses and the interlobular bile ducts, although not a strict localization to the canals of Hering. According to Ribadeau-Dumas and Lecène,[78] the distribution of these lesions corresponds to the biliary lobules of Sabourin adjoining the portal canals.

Necrosis in the inner third of the lobule has been attributed to overloading of the cells with bile (Ogata [15]), which is thought to be secreted more abundantly in this area (Bürker [25]). The degree of pigmentation by bilirubin, which is nontoxic, is no indication, however, of the presence of toxic components of bile, although deleterious changes may conceivably ensue on actual physical overloading with this inert substance. There is abundant evidence suggesting the presence in the liver affected by stasis of a toxic factor in escaped bile which may affect chiefly the inner cells of the lobule, for when both central and peripheral cells are subjected to a known irritant under identical conditions, the former succumb much more readily and show the most marked degree of

74. Chrzonsczczewsky, N.: Virchows Arch. f. path. Anat. **35**:153, 1866.

75. Heidenhain, R. P. H.: Handbuch der Physiologie, Leipzig, F. C. W. Vogel, 1883, vol. 5, p. 225.

76. Stern, H.: Arch. f. exper. Path. u. Pharmakol. **19**:39, 1885.

77. Pfeiffer, L.: Arch. f. mikr. Anat. **23**:22, 1884.

78. Ribadeau-Dumas and Lecène: Arch. d. méd. expér. et d'anat. path. **16**:191, 1904.

damage (Simonds and Callaway [79]). This vulnerability of the centrally located hepatic cells has been attributed to their greater functional activity and higher degree of specialization (Mallory [80]).

There was little or no attempt at regeneration. Multinucleated hepatic cells were rarely found, and mitotic figures and evidence of nuclear fission were not observed. Areas of destruction of the hepatic cells were gradually absorbed but were not replaced by new cells. These observations are in keeping with experimental data on the failure of restoration of the liver following obstruction of the common bile duct (Mann, Fishback, Gay and Green [81]).

Early in obstructive jaundice the proliferation of bile ducts and the increase in connective tissue are intimately related. As the newly formed bile ducts invade the peripheral portions of adjacent lobules they carry along a covering of connective tissue which enlarges the portal radicle. Steinhaus,[65] Gerhardt,[36] Chambard,[33] Foà and Salvioli,[56] Pick,[57] Rous and Larimore [59] and Richardson [55] attributed the changes in the bile ducts to the irritation arising from increased pressure within them. Charcot and Gombault,[5] Nasse [82] and Ogata [15] considered the two processes to be essentially independent although brought about by the same cause, namely, the irritation from static bile. We agree with Harley and Barratt,[83] Loeffler [63] and Paolini [84] that the changes in the bile ducts and connective tissue are probably brought about, at least in part, by a combination of both of these factors. As the pressure within the bile ducts increases there are a coincident stretching and attenuation of their walls, and bile diffuses into the surrounding tissue, where it actively stimulates hyperplasia of the connective tissue (Richardson [55] and Rous [28]), which is only slight or moderate within the first two months but then proceeds independently of the changes in the bile ducts. It is possible that the damaged hepatic cells form intermediate metabolic products the action of which also stimulates the growth of connective tissue. A number of investigators advanced the view that the proliferation of the connective tissue depends largely on the organization of necrotic foci (Beloussow,[64] Foà and Salvioli,[56] Gerhardt,[36] Janowski,[8] van Heukelom,[85] Tsunoda,[58] Purpura [86] and Fabris [87]). In our cases, the focal midzonal necroses showed

79. Simonds, J. P., and Callaway, J. W.: Am. J. Path. **8**:159, 1932.

80. Mallory, F. B., cited by Simonds and Callaway.[79]

81. Mann, F. C.; Fishback, F. C.; Gay, J. G., and Green, G. F.: Arch. Path. **12**:787, 1931.

82. Nasse, D.: Semaine méd. **16**:202, 1894.

83. Harley, V., and Barratt, W.: Brit. M. J. **2**:1743, 1898.

84. Paolini, R.: Riforma med. **43**:843, 1927.

85. van Heukelom, S.: Beitr. z. path. Anat. u. z. allg. Path. **20**:221, 1896.

86. Purpura, F.: Sperimentale, Arch. di biol. **69**:819, 1915.

87. Fabris, A.: Arch. per le sc. med. **31**:429, 1907.

no attempt at organization, although the reactive and healing processes about the biliary necroses did contribute to the new formation of connective tissue at a time, however, when that resulting from other causes was already well established.

SUMMARY

With obstruction to the common bile duct by carcinoma of the head of the pancreas, the biliary conducting system undergoes a tremendous volumetric increase, with corresponding stretching and thinning of its walls. The process extends uniformly into the branches of the fourth and fifth orders, although the dilatation may be more marked in the radicles of the left hepatic lobe by virtue of the lessened amount of parenchyma in that portion of the liver and also possibly because of the course and relationship of the left hepatic duct and its corresponding artery and vein. The vasa aberrantia and parietal sacculi become effaced. The contents of the biliary conducting system in the beginning consist of dark, thick, ropy, inspissated bile which later tends to become pale and thinner. The canaliculi are distended with bile thrombi, many of which remain in situ, although others are extruded into the tissue spaces and sinusoids, where they may be phagocytosed by Kupffer cells and macrophages. Bile pigment in the form of granules or droplets and in colloidal suspension may be found in varying amounts, chiefly in the cells of the central portions of the lobule. Regressive lesions are present as mild and localized degenerative and necrotic changes involving the hepatic cells about the central vein, as nonpigmented focal midzonal areas of necrosis and as deeply pigmented biliary necroses occurring either in the outer portion of the lobule or within the portal radicle. The intrahepatic ducts elongate and become tortuous, and they show, in addition, a true proliferation. A new formation of connective tissue occurs simultaneously with the changes in the bile ducts until after the second month, when a rapid, independent and progressive proliferation takes place, resulting in a well marked deposit of connective tissue which is interlobular, intralobular and, in long-standing cases, even perilobular in distribution. The hepatic lobules show a corresponding reduction in size without much architectural alteration except at the peripheries, where atrophy may become well marked. The walls of the branches of the hepatic artery undergo hypertrophy, and those of the branches of the hepatic vein and to a less extent of the portal veins show a new formation of fibrous tissue. The liver possesses little or no regenerative ability in the face of total stasis.

INFLUENCE OF EXTRACT OF ANTERIOR PITUITARY ON AUTOTRANSPLANTED AND HOMEO-TRANSPLANTED THYROID

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Loeb and his collaborators [1] showed that extracts of the anterior lobe of the pituitary gland stimulate the growth of the thyroid. This effect is especially marked in guinea-pigs. These observations were confirmed by many other investigators.[2]

In another series of investigations, Loeb [3] analyzed the reactions of the host against autotransplants, syngenesiotransplants, homeotransplants and heterotransplants. Definite relations were found to exist between the intensity of the lymphocytic and connective tissue reactions on the part of the host and the genetic relationship of the host and the donor. While autotransplantation in general makes possible the normal development of the graft, homeotransplantation, as a rule, leads to severe injury or complete destruction of the transplant.

It seemed of interest to investigate the behavior of the various types of transplants under the influence of extract of the anterior lobe of the pituitary gland of cattle. The thyroid gland represents an especially favorable material for such investigations. In particular did I wish to answer the following questions:

1. Under the influence of the extract does the transplant change similarly to normal thyroid tissue?

2. Does this change have any effect on the resistance of the transplant to the direct and indirect action of homeotoxins?

I therefore carried out two series of experiments on guinea-pigs; in the first, the effect of this extract on the growth of autotransplants was studied, and in the second, its effect on the reactions of the host against homeotransplants of thyroid tissue and on the preservation of

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1. Loeb, L.: Klin. Wchnschr. **11**:2121, 1932. Loeb, L., and Bassett, R. B.: Proc. Soc. Exper. Biol. & Med. **26**:860, 1929; **27**:490, 1930. Loeb, L.; Bassett, R. B., and Friedman, H.: ibid. **28**:209, 1930.

2. Oehme, C.; Poal, H., and Kleine, H. O.: Klin. Wchnschr. **11**:1449, 1932. Schittenhelm, A.: Deutsche med. Wchnschr. **58**:803, 1932. Silberberg, Martin: Krankheitsforschung **8**:171, 1930; Proc. Soc. Exper. Biol. & Med. **26**:166, 1929; Virchows Arch. f. path. Anat. **289**:201, 1933.

3. Loeb, L.: Am J. Path. **2**:99 and 315, 1926; **3**:251, 1927; J. Cancer Research **10**:252, 1926; Am. Naturalist **54**:45, 1920; J. Gen. Physiol. **8**:417, 1926; Endocrinology **13**:49, 1929; Physiol. Rev. **10**:547, 1930; Biol. Bull. **40**:143, 1921.

the latter. Altogether, seventy-four animals were used in these experiments. I shall describe only certain representative experiments among a larger number which I have carried out.

I used acid extract of the anterior lobe of the pituitary gland prepared in a similar way to that used in my previous investigations, except that a small piece of camphor was added to the extract as a preservative. One and one-half cubic centimeters of this extract was injected daily into guinea-pigs intraperitoneally. Young guinea-pigs weighing on the average from 150 to 200 Gm. were used, and the changes in weight of the animals were determined each day before feeding. The experiments were carried out mainly during the autumn and winter. As a rule, one lobe of the thyroid gland was transplanted into a subcutaneous pocket in the abdominal wall of the host animal, with aseptic precautions, under narcosis with ether.

At the conclusion of the experiment, the transplant was taken out together with the surrounding host tissue and fixed in a trinitrophenol-formaldehyde solution.[4] This material was embedded in paraffin and cut in serial sections, which were stained with hematoxylin and eosin.

OBSERVATIONS

I. *Effect of the Extract on the Normal Thyroid* (six experiments).—As described by various investigators, daily injections of from 1 to 3 cc. of this extract produced an enormous increase in the size and number of cells of the acinar epithelium, the proliferation taking place by means of mitoses. The first changes were found after one injection. The strongest effect, however, was seen on an average after from six to ten injections: The formation of large papillary excrescences protruding into the lumen of the acini, migration of phagocytes into the colloid, softening, liquefaction and perfect absorption of the colloid were observed. Under these conditions, the thyroid assumed the appearance of that characteristic of exophthalmic goiter.

II. *Effect of the Extract on the Autotransplanted Thyroid.*—1. Controls (eight experiments): After autotransplantation of the normal gland, the peripheral part of the thyroid remained alive, while the insufficiently nourished central portion became necrotic and was replaced by dense connective tissue. In the living part as well as in the adjoining portion of the central necrotic zone, there was a new formation of blood vessels. Fibroblasts and histiocytes grew into the central necrotic mass and absorbed it. Within about three weeks, regeneration of the acini was taking place from the peripheral zone, and the transplant more and more assumed the appearance of the normal thyroid. The colloid was hard. In some cases a loose, well vascularized connective tissue was found in the center of the transplant instead of acini. The surrounding tissue likewise consisted of a more or less loose connective tissue, which was well vascularized.

4. This solution is composed of: solution of formaldehyde, U. S. P., 500 cc.; distilled water, 1,500 cc.; glacial acetic acid, 100 cc.; trinitrophenol to saturation point.

2. Autotransplanted Thyroid: GROUP 1.—In this group autotransplantation of the thyroid gland was followed by a series of injections of the extract with and without intervening periods of rest. In other experiments, the series of injections was either preceded or followed by a period of rest, during which no injections were given.

(*a*) Autotransplantation, followed by six injections of extract, one being given daily (two experiments). The transplanted thyroid showed hypertrophy and hyperplasia. The effect of the extract was evident in a larger number of mitoses, the increased size of the acinar epithelium, the presence of some phagocytes in the acini and a softening and liquefaction of the colloid. A considerable increase in the number of acinar cells had taken place. It was interesting that in comparison with the controls, the central portion of the transplant seemed to be better preserved, indicating a greater resistance of this tissue.

(*b*) Autotransplantation, followed by six injections of extract and a subsequent period of rest. In the transplants which were removed six days after the last injection (two experiments), the effect of the extract on the transplant as well as a good regenerative growth were still observed. But the acinar epithelium had become lower and the number of the mitotic figures was diminished; the thyroid transplant was on the way to resuming the structure of the normal, resting gland.

After twelve days of rest (two experiments), signs were still present of the preceding period of hypertrophy in the transplanted tissue, which had healed in well; the regenerative growth had been very good. The acini were increased in number; they were still enlarged and contained again hard, newly formed colloid. The periphery was surrounded by loose connective tissue; a central fibrous knob was not visible.

(*c*) Autotransplantation, followed by a period of rest lasting five days, then by six subsequent injections of extract (four experiments). The transplant was very well preserved. The formerly necrotic central zone had been replaced by dense fibrous tissue. In the peripheral area regeneration was noticeable in addition to the typical hypertrophy caused by the extract.

2. Transplantation, followed by a period of rest lasting fifteen days, then by ten subsequent injections, one being given daily (two experiments). In this case in which the injections were given after a longer period of rest following transplantation, at a time therefore when the transplant had regained the structure of the normal thyroid, the effects of the extract were the same as in the normal thyroid. The graft showed a hypertrophic acinar epithelium, increase in mitotic proliferation and liquefaction or complete absorption of the colloid.

3. Transplantation, followed by a period of rest lasting eighteen days, then by a series of six injections (two experiments). Under the conditions of this experiment the hypertrophy was more marked than under those of the preceding experiment. Figure 1 shows the typical structure. The thyroid was well healed in and was surrounded by a small amount of connective tissue and large blood vessels. In the transplant itself marked hypertrophy was observed. The cells of the acinar epithelium were large and high; there was an increased number of mitoses, and the colloid had become soft and had mostly been absorbed; owing to the increase in the number of acini, the interacinar connective tissue had almost completely disappeared.

Summary of series *c*: The influence of the extracts on the transplants was striking. If a period of rest was maintained between transplantation and the beginning of the injections, the hypertrophy of the acini was more marked than

in those experiments in which injections followed the transplantation directly. However, the difference between these two types of experiments is only of a quantitative character.

(*d*) Transplantation, followed by a period of rest lasting six days, then by six injections of extract, followed by a second period of rest. The only difference between the results in this group of experiments and those in the preceding group consisted in the beginning disappearance of the effects of the extract when injections were omitted for some time previous to examination. This applies especially to the character of the colloid; a new formation of hard colloid had set in under these conditions. However, an increase in size and number of the acini was still

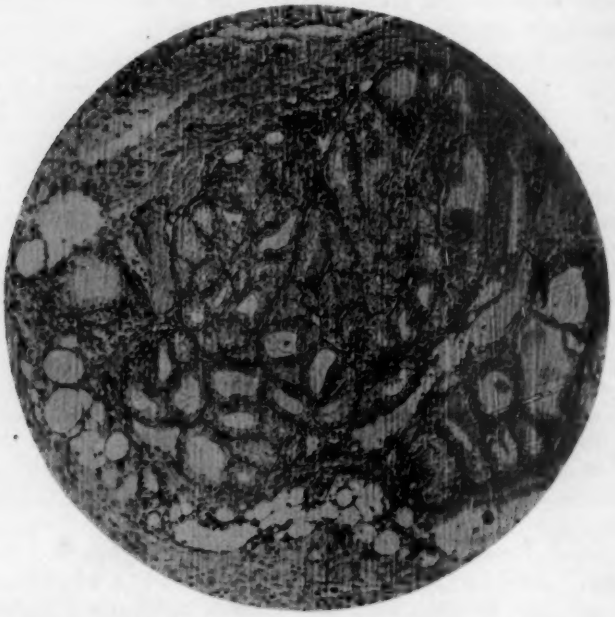

Fig. 1.—Autotransplantation was followed by eighteen days of rest and six injections of extract of the anterior lobe of the pituitary gland. The acini are free from colloid, the epithelium enlarged and a capsule of connective tissue surrounds the transplant. Medium magnification.

noticed, but the epithelium had become lower and the mitotic figures were diminished in number. The thyroid was therefore in the process of returning from a state of hypertrophy to that of rest.

Group 2.—In this group, transplantation was preceded by a series of injections of extract of the anterior lobe of the pituitary gland; subsequent to transplantation there was a period of rest.

(*a*) Six injections of extract, followed by transplantation and a six day period of rest (six experiments). The central portion of the transplant was necrotic, while the peripheral part of the gland was preserved. The surrounding loose fibrous tissue contained a few fibroblasts and blood vessels. Under high power magnification one could still notice a certain effect of the extract, as the colloid was softened

and contained some phagocytes, and many mitotic figures were seen, but the acinar epithelium had already become lower and was in the process of returning to a state of rest.

(*b*) Ten injections given previous to transplantation, with a period of rest lasting ten days between transplantation and examination (two experiments). During the ten day period of rest a return of the graft to a normal condition was noticeable; the phagocytes had disappeared and though there was still an increased number of mitoses, the acinar epithelium had become flatter.

(*c*) Six injections, followed by transplantation and a period of rest of eighteen days. In this transplant, the necrotic part had been replaced by a dense fibrous tissue. From the peripheral remnant of the thyroid, regeneration had developed. The changes, due to a preceding stimulation of growth by the extract, had disappeared. A new formation of hard colloid in the well formed acini had taken place.

GROUP 3.—A series of injections was followed by transplantation; the graft was then subjected to a new series of injections. The transplant was removed either directly after the last injection or following a period of rest subsequent to the second period of injections.

(*a*) Six injections, followed by transplantation and a second series of six injections (four experiments). A small central area of the transplant was still partly necrotic, but its organization by fibrous tissue had progressed. The periphery was well formed and showed large acini with hypertrophic cells. The colloid was softened or entirely liquefied. The softened colloid might have contained phagocytes. Regeneration, as usual, had started from the peripheral living thyroid tissue.

(*b*) Six injections, followed by transplantation, then a second series of eighteen injections (two experiments). The central, formerly necrotic zone of the thyroid was replaced by a dense fibrous tissue. In the periphery the stimulation was indicated by the finding of a large number of mitoses, but the stimulation was not so marked as after six injections, as indicated by the lower acinar epithelium in comparison with the earlier stage. In the acini only a few phagocytes were visible.

(*c*) Six injections, followed by transplantation, then a second series of six injections. The thyroid was examined following a period of rest lasting eighteen days. The regeneration of thyroid tissue had made good progress, but the acinar epithelium was in the process of returning to the resting stage, as indicated by new formation of hard colloid.

(*d*) Six injections followed by transplantation, then by an eighteen day period of rest and a second series of six injections (two experiments). The regeneration was completed. The peripheral acini were distended and showed the structural changes characteristic of the extract. However, the growth was not so good as in those cases in which the animal did not receive injections before transplantation.

III. *Effect of the Extract on the Homeotransplanted Thyroid.*—After determining the effect of the extract of the anterior lobe of the pituitary gland on the autotransplanted thyroid, it was of interest to determine the effect of this substance on the homeotransplanted gland. The fate of the homeotransplanted thyroid gland differs greatly from that of the autotransplanted tissue. The various processes leading to the destruction of the former have been analyzed by Hesselberg and Loeb. Some control experiments were first carried out in which homeotransplantation of the thyroid gland of the guinea-pig was carried out in order to obtain a standard with which to compare the behavior of the homogeneous graft under the influence of extracts of the anterior lobe of the pituitary gland.

1. Controls (four experiments): Connective tissue grew in greater quantity into the central necrotic part of the homeotransplanted than of the autotransplanted gland, and it destroyed the peripheral, living, glandular zone, first causing a shrinkage and gradual obliteration of the acinar lumens. Seven or eight days after transplantation, a remarkable infiltration of the transplant with lymphocytes developed and helped in the destruction of the transplant. Usually from twenty to thirty days after transplantation the graft was completely destroyed. As a rule, homeotransplants are preserved for only a short time. While regenerative processes may be found also in homeotransplants, they are generally more extensive in autotransplants. Twenty-four days following homeotransplantation, a perfect healing in of thyroid tissue or a new formation of acini with colloid such as was seen in the autotransplanted glands had not taken place.

Furthermore, it may be noticed that it is nearly always the lymphocytes which mainly cause the elimination of the homeotransplant, while in autotransplants these cells are present only in a very small number, and in later periods they are not more frequent than in the normal gland.

2. Experiments with Extract: Group 1.—Homeotransplantation was followed by injections of extract.

(*a*) Homeotransplantation, followed by eighteen injections of extract, then removal of the transplant for microscopic examination (two experiments). The peripheral part, as well as the center of the transplant, showed preserved thyroid tissue. The surrounding area consisted of relatively dense fibrous tissue. A regeneration of acini had taken place. It was especially noted that a mass of lymphocytes penetrated the transplant without, however, completely destroying it. The effect of the extract was clearly indicated by the high acinar epithelium, the large number of mitotic figures and the softened or liquefied colloid; the change in the homeotransplant was, therefore, similar to that called forth in the normal gland by the injection of the extract. However, after eighteen injections the normal thyroid does not usually show so strong an influence of the extract as did this transplant.

(*b*) Homeotransplantation, followed by a series of eight injections, then by a ten day period of rest (two experiments). The principal difference between this and the former experiment was that here only very few acini were preserved. The main part of the transplant was destroyed by lymphocytes; a newly formed capsule of connective tissue was not observed. The only part of such transplants preserved may consist of a few scattered acini. In another similar experiment the whole transplant had been destroyed.

(*c*) Transplantation, followed by a period of rest lasting five days, then by a series of twelve injections, one being given daily (two experiments). In this experiment the whole transplant was well preserved and enclosed in a dense fibrous capsule in one animal. In the periphery was found excellently regenerated thyroid tissue in which some acini contained colloid. In the center some lymphocytes were found, which, however, did not cause much destruction. Under high power magnification the effect of the extract was recognized by the presence of high acinar epithelium, an increase in the number of mitotic figures and the liquefaction of the colloid containing a few phagocytic cells. However, as far as the regeneration of the transplanted tissues in the two animals used in this case was concerned, considerable variations were found; while one of the transplants was well preserved, the other did not show any growth, although both pieces had been left in their respective hosts for the same length of time and an exchange of thyroids had taken place between the two animals.

(*d*) Transplantation, followed by a five day period of rest, then a series of six injections followed again by a seven day period of rest (two experiments). The center of the transplant consisted of a small amount of connective tissue with many lymphocytes, but also in this case the transplant was, on the whole, well preserved. The effect of the extract was diminishing: the epithelium of the acini became lower and the colloid accumulated again in the acini. In the periphery some lymphcoytes were seen, and the surrounding area consisted of fibrillar connective tissue and blood vessels. There were no homeotoxic cells, which destroy the transplant. Growth and regeneration in this experiment were very good and similar to those seen in series *c* (fig. 2).

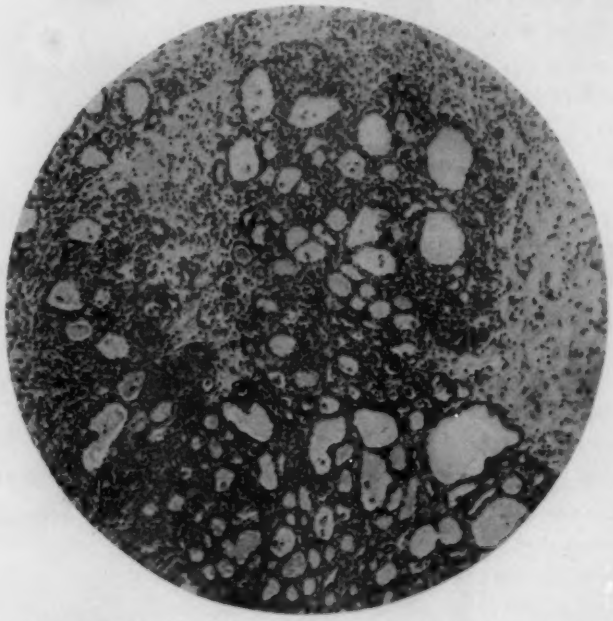

Fig. 2.—Homeotransplantation was followed by five days of rest and six injections, and a second period of rest lasting seven days. Connective tissue and enlarged acini are seen. Medium magnification.

GROUP 2.—A series of six injections was given to the donor of the transplant preceding the transplantation. As a result of these injections, the thyroid to be transplanted had become hypertrophic. The hypertrophic gland was then transplanted. The graft was removed at the end of the experiment, either following a period of rest or after a series of injections had been given to the host, so that the transplant was subjected altogether to the influence of two series of injections of extract, one being given before transplantation and a second one following transplantation.

(*a*) Six injections given to the donor; transplantation of the hypertrophic gland, followed by a period of rest of eighteen days (two experiments). In this series only small remnants of the transplants were found. Almost the whole thyroid transplant was destroyed and replaced by connective tissue containing a large

number of lymphocytes. No difference between these pieces and the control homeotransplants could be seen.

(*b*) Six injections given to the donor; transplantation, followed by six injections into the host (three experiments). The transplant was necrotic in the center. A loose fibrous tissue developed here, and lymphocytes began to invade the transplant. There was no essential difference between the results of these experiments and those of the controls.

(*c*) Six injections given to the donor; transplantation; six injections into the host followed by twelve days of rest (two experiments). The results are the same as in experiment *b*. The graft was completely destroyed by lymphocytes. No regeneration had taken place, nor was an effect of the extract noticeable (fig. 3).

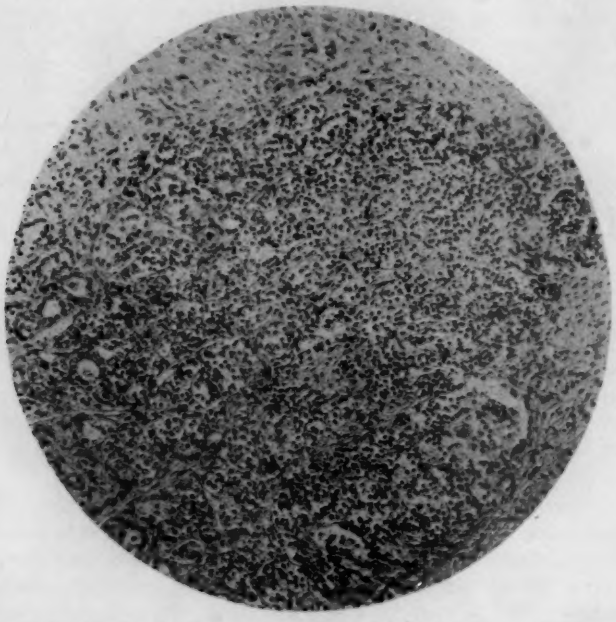

Fig. 3.—The thyroid used for homeotransplantation was taken from a donor which had received six injections with the extract. The new host received six injections; after a subsequent period of rest lasting twelve days, the homeotransplant was examined. The greater part of the graft has been destroyed. Medium magnification.

(*d*) Six injections given to the donor; transplantation, followed by eighteen injections into the host (two experiments). Under the conditions of this experiment the graft was likewise completely destroyed by homeotoxic lymphocytes.

A number of other similar experiments had analogous results.

COMMENT

My results are in agreement with the conclusions of Loeb and of Borst,[5] who showed that the thyroid gland represents a relatively resistant tissue and that autotransplantation of this organ is successful.

5. Borst, M.: Brit. M. J. **2**:383, 1913.

They agree also with the view that the relations between the genetic constitutions of the host and the donor, as expressed in the individuality differentials, determine the result of transplantation. My experiments have shown that the transplanted organ responds to the stimulus of the hormone of the anterior lobe of the pituitary gland in approximately the same manner as does the normal organ, and this applies to the autotransplant as well as to the homeotransplant, although in the case of the latter the amount of tissue that remains alive is much smaller and the parts that remain alive are exposed to the injurious reactions of the host. As Loeb has shown, compensatory hypertrophy may develop in autotransplanted as well as in homeotransplanted thyroids. Likewise, retrogression of the hypertrophic and hyperplastic changes in the transplants following the cessation of injections of the extract of the anterior lobe of the pituitary gland takes place in the same manner in the transplants as in the normal gland which has been left in situ. Thus, while the transplants are affected by the extract in a similar way to the normal gland, the hypertrophic and hyperplastic changes may not be quite so marked in the transplants, perhaps owing to the relatively unfavorable conditions which are present in the graft during the period following transplantation. A summation of the stimulus to regenerative growth which acts on the transplant, owing to its separation from its normal environment, and of the effect of the extract does not take place.

The result is different when compensatory hypertrophy is induced in one of the lobes of the thyroid gland through extirpation of the other lobe; if extract of the anterior lobe of the pituitary gland is allowed to act on thyroid tissue which is in process of compensatory hypertrophy, a summation of the two effects, compensatory hypertrophy and hypertrophy and hyperplasia induced by the extract, does occur, presumably because under these conditions the nontransplanted tissue is in a satisfactory state and can readily respond to the stimulating agent.

As stated, extract of the anterior lobe of the pituitary gland can exert a stimulating effect also on the homeotransplanted thyroid gland, and it is probably due to the increased growth energy induced in the transplant by the hormone of the anterior lobe of the pituitary gland that the thyroid tissue is able to maintain itself better than the ordinary homeotransplanted gland and to overcome to some extent the injurious action of the lymphocytes of the host. In order to accomplish this result, the injections of the extract must be begun at an early period following transplantation; otherwise, if the injections are started at a time when the reaction against the transplant has become manifest, the stimulation by the hormone of the anterior lobe of the pituitary gland is without avail. If, in some cases, the graft is destroyed at an early period, notwithstanding the large doses of extract given, one may assume

that the difference in the constitution of the individuality differentials of host and transplant was so great that it could not be overcome by the added growth momentum of transplanted tissue.

One might suppose that the regenerating tendency of thyroid tissue, which had been exposed to the influence of the extract previous to transplantation, would be very strong, but, under these conditions, the tissue to be transplanted is already hypertrophic, and hypertrophic thyroid tissue seems unable to withstand the injurious effects of either autotransplantation or homeostransplantation as readily as normal thyroid gland. One may assume that the hypertrophic tissue is much more active, as far as metabolic changes and growth are concerned, than the ordinary thyroid gland, and, furthermore, that it needs a greater amount of oxygen than can be supplied during transplantation; thus it is injured to a greater extent than is the normal thyroid gland. One finds, therefore, that whereas administration of extract of the anterior lobe of the pituitary gland following transplantation exerts a beneficial effect on the transplanted tissue, administration of this extract previous to transplantation is very injurious and leads to more unfavorable results than transplantation of the normal organ.

SUMMARY

1. Injection of extracts of the anterior lobe of the pituitary gland may induce hypertrophic and hyperplastic changes not only in the normal, but also in the transplanted, thyroid gland of the guinea-pig; it may be effective not only in the autotransplanted, but also in the homeotransplanted, gland. However, the effect is usually less marked in the transplant than in the normal tissue.

2. The stimulation of the homeotransplanted thyroid tissue by the hormone of the anterior lobe of the pituitary gland may increase the ability of the graft to resist the injurious factors which are active after homeotransplantation; the preservation of the graft is thus improved. In order to accomplish this effect, it is necessary to apply the extracts after the thyroid has been transplanted into the strange host.

3. If, however, the tissue is caused to grow and to undergo hypertrophic changes previous to transplantation, the thyroid tissue shows a diminished power of resistance and is destroyed at an early period following transplantation. One must assume that the stimulated thyroid gland is less suitable for transplantation than the resting normal gland.

General Review

MELANIN

II. A REVIEW OF CHEMICAL ASPECTS OF THE MELANIN PROBLEM

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The classification of the many colored materials found in human tissues in health and in disease presents problems which have been approached from various angles over a long period of years. The problems as a whole are similar for the lower orders of the animal kingdom as well, and possibly even for the protozoa. The medical student is taught that the pigmented substances of the body are divided into two main groups, the endogenous and the exogenous. The former consist of the pigments derived from hemoglobin, such as hemosiderin, hematoidin, hemofuscin and the bile pigments, the rather indefinite group of lipochromes and melanin. The exogenous pigments come from inhaled dust, producing the pneumonokonioses, from tattooing and from foods and medicinal substances, causing carotenemia and argyria, for instance.

Melanin has generally been regarded as strictly endogenous, but since it is found in foods of plant and animal origin, it has also, at least theoretically, these exogenous sources. The term melanin is derived from the Greek μέλας, meaning black. The pigment is black, however, only when viewed in large amounts. Smaller deposits are brownish, and hence fuscin would be more appropriate etymologically. In the medical literature are found such expressions as melanism or melanosis, meaning varying degrees of melanin deposit in the skin; melanocyte, a cell containing melanin; melanoma, a tumor containing melanin, and melanuria, the presence of melanin in the urine.

Melanin is found in the pigment of the hair, the skin, the choroid of the eye, the ciliary body, the pigment layer of the retina and various nerve cells. There are analogous foci of coloration in the lower animals, the "ink" of the squid and butterfly wings; in fact, the entire animal kingdom uses this pigment in its manifold color patterns and for protection

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by color mimicry against environmental perils. The amount of melanin necessary to color tissues grossly is very small. It has been estimated that there is only 1 gram in the entire skin of a Negro (Abel and Davis [1]). In a liver affected with metastatic melanoma, Berdez and Nencki [2] obtained 300 Gm. of melanin and estimated 500 Gm. in the entire body. Helman [3] computed that melanin may constitute 7.3 per cent by weight of the fresh substance of some melanomas. The smallest amount of melanin or its total absence is seen in the albino, a congenital condition which occurs as a mendelian recessive character. Acquired loss of melanin usually occurs in localized areas of the body, the best example of this being vitiligo. However, in alopecia areata, leprosy, tuberculids, syphilids and burns, the lesion is often associated if not in the most active stage, at least in the healing and healed lesions, with partial or complete loss of the pigment in the skin and in the hair. Indeed, it seems that every disease of the skin has an effect on its melanoblasts and melanophores, but the effect may be either loss of pigment or a great increase and is dependent on factors little understood.

Increased melanin deposits are seen in a variety of conditions. The normal melanin content varies with the races, with racial subdivisions and even individually, so that no color standard is possible. From ordinary "tanning" and the freckle or lentigo, chloasma or liver spots to xeroderma pigmentosum, pigmented papillomas and the pigmented nevus, there is at the other end of the spectrum the very dangerous tumor melanoma malignum. As expressions of serious disease involving the pigment regulatory system there are Addison's disease, Recklinghausen's disease (neurofibromatosis), arsenic poisoning and senility, in which diffuse melanosis cutis is often a striking feature. Endocrine disturbances affecting especially the anterior lobe of the hypophysis and the thyroid are often accompanied by excessive pigmentations.

A substance with so definite a name which has adhered for these many years naturally has been studied to determine its chemical composition. The greatest difficulty in the way of such an investigation is its relative insolubility in fluids from which it could be extracted in pure form through crystallization. Melanin can be removed from tissues with alkalis and to a less degree by acids, but while the resultant solution is highly colored it cannot be assumed that the extracted material is any longer simply melanin. Nevertheless, Hoffmeister [4] endeavored to

1. Abel, J. J., and Davis, W. S.: J. Exper. Med. **1**:361, 1896.

2. Berdez, J., and Nencki, M.: Arch. f. exper. Path. u. Pharmakol. **20**: 346, 1886.

3. Helman, D.: Zentralbl. f. inn. Med. **23**:1017, 1902.

4. Hoffmeister, cited by Wells, H. G.: Chemical Pathology, Philadelphia, W. B. Saunders Company, 1925, p. 527.

establish its molecular composition and arrived at the proportion N:H:C = 1:5:5. Sulphur was high, about 10 per cent in melanosarcomas and 12 per cent in the ink of the squid. No sulphur was found in choroid melanin. The sulphur in hair was from 2 to 4 per cent. A trace of iron was sometimes found. Salkowski [5] mentioned several melanins all of which contain sulphur and phosphorus. As stated by Matthews,[6] the composition of melanin is not definitely known, and he regards it as a not well defined chemical substance.

Gortner,[7] who has studied plant and animal pigments for many years, gave the following composition for melanin: C, 52.57: H, 7.28: N, 13.43: S, 1.33, or, comparing his findings with Salkowski's ratio of N:H:C = 1:5:5, the proportions would be N:H:C = 2:1:7.5. This divergence probably is due to the different sources of the pigments used. It does not, however, prove that there is not a basic pigmented component of the complex biologic unit called melanin.

Gortner [8] isolated from black wool by treatment with extremely dilute alkalis a material of protein nature which was intensely black but which on acid hydrolysis yielded about 90 per cent of its weight in amino-acids. This complex he regarded as a conjugated protein in which melanin is the associated chromogen. He was unable to isolate a similar compound from nonpigmented wool, and since other pigmented structures like human hair, black rabbit fur and black feathers did not yield a similar product, he thought it reasonable to believe that the pigment of black wool exists in the form of melanoprotein rather than as melanin unassociated with the protein molecule. Spiegler,[9] on the other hand, isolated from white wool a white chromogen closely related to melanin chemically.

The animal pigment melanin is now generally regarded as formed by the interaction of a chromogen and an oxidase which is apparently tyrosinase.

In 1896 Bertrand [10] discovered an oxidase which produced various colors to a black precipitate with tyrosine and called it tyrosinase. In 1902 von Fürth and Schneider [11] suggested that melanins were probably the product of the interaction of tyrosine and tyrosinase. In the same year Dewitz [12] found that pigmentation of the fly, Lucilia

5. Salkowski, E.: Virchows Arch. f. path. Anat. **240**:353, 1922.

6. Matthews, A. P.: Physiological Chemistry, New York, William Wood & Company, 1925, p. 710.

7. Gortner, R. A.: J. Biol. Chem. **8**:341, 1910.

8. Gortner, R. A.: Biochem. Bull. **1**:207, 1911.

9. Spiegler, E.: Beitr. z. chem. Physiol. u. Path. **4**:40, 1902; **10**:253, 1907.

10. Bertrand, G.: Compt. rend. Acad. d. sc. **122**:1215, 1896.

11. von Fürth, O., and Schneider, H.: Beitr. z. chem. Physiol. u. Path. **1**: 229, 1902.

12. Dewitz, M. I.: Compt. rend. Soc. de biol. **54**:44, 1902.

caesar, was caused by the action of tyrosinase on a chromogen. In 1900 Roques [13] showed that a similar reaction occurred during metamorphosis and pigmentation of a beetle, Limnophilus flavicornis, Fabr., and in 1905 Phisalix [14] noted similar changes in the integument of the cockroach larva, Phyllodromia germania. In 1904 Durham [15] extracted the fetal skins of black rabbits and agouti guinea-pigs, and after incubating at 37 C. for ten days in the presence of tyrosine and ferrous sulphate dark precipitates formed. He used toluene as a preservative, and no coloration was produced without the use of ferrous sulphate.

Gortner [16] noted two varieties of tyrosinase in the meal worm, Tenebrio molitor, one water-soluble, the other water-insoluble, the latter in greater amount. The enzyme is uniformly distributed, but the color pattern in the potato beetle, for instance, was thought to be due to a localized secretion of chromogen. In his analyses published in 1911 Gortner [17] found that melanin on hydrolysis yielded tyrosine, lysine, arginine and 10 per cent "melanohumin," a brownish-black residue. Hence he regarded melanin as essentially of protein nature, a conclusion supported by the recent immunologic studies of Adant [18] at the University of Louvain. The inability of chemists to isolate the basic pigmented factor has led to the use of the term melanins, suggesting that their number may be legion. Gortner [17] stated that all melanoproteins are melanin, but not all melanins are melanoproteins. Some are acid-soluble. Those of keratin are insoluble in alkali until some decomposition has set in. The melanoproteins stain keratin diffusely as in auburn hair. Gortner further found that *m-di*-hydroxyphenols (orcinol, resorcinol and phloroglucinol) inhibit the oxidation of tyrosine by tyrosinase, yet do not destroy the tryrosinase, nor are they themselves oxidized in preference, their presence being sufficient to inhibit the reaction. While no similar chemical has as yet been isolated from a dominant white organism, this test tube experiment is at least suggestive of the mechanism that may be involved. Dominant whites, he holds, are due to the presence of anti-oxidase, while regressive whites have not the power either to form pigments or to inhibit their formation.

The old idea that melanin is derived from hemoglobin no longer has adherents. Mierowsky's [19] studies with incubated skin were strongly against this hypothesis. He found also a pyronine-red substance in

13. Roques, X.: Compt. rend. Acad. d. sc. **149**:418, 1909.
14. Phisalix, M. C.: Compt, rend. Soc. de biol. **57**:17, 1905.
15. Durham, F. M.: Proc. Roy. Soc., London **74**:310, 1904.
16. Gortner, R. A.: J. Biol. Chem. **10**:89, 1911.
17. Gortner, R. A.: Am. Naturalist **45**:743, 1911; J. Biol. Chem. **10**:113, 1911.
18. Adant, M.: Arch. internat. de méd. expér. **7**:698, 1932.
19. Mierowsky, E.: Ztschr. f. Path. **2**:438, 1909.

cell nuclei, and this substance Dyson [20] thought is the chromatinprotein part of the melanin granule.

Piettre [21] hydrolyzed animal melanin with 25 per cent sulphuric acid and obtained amorphous amino-acids, tyrosine, leucine and a melanotic pigment. This pigment was insoluble in acids but very soluble in alkali. Melanin easily loses ammonia in alkaline solution.

Young,[22] in studying the pigment in the skin of an Australian black, extracted it in warm 5 per cent potassium hydroxide. The filtrates on cooling deposited much fat, which was removed. The final clear filtrate was acidified with hydrochloric acid; the pigment was precipitated as a dark brown powder, redissolved repeatedly in 5 per cent potassium hydroxide, filtered and reprecipitated and thoroughly washed with distilled water, dried, washed with alcohol, ether and carbon disulphide and dried in a vacuum over sulphuric acid. This powder was quite hydroscopic. Analyses showed it to contain carbon, hydrogen, nitrogen and sulphur and a trace of iron. When tested in solution spectroscopically, it was found that all rays in the violet, blue and part of the green were absorbed to a wavelength of about 515 microns.

Fürth and Schneider [11] regarded melanin as depending on the action of tyrosinase in conjunction with autolytic enzymes, the latter being freed from the chromogen groups of the protein molecule and then oxidized by the tyrosinase, undergoing condensation and taking up sulphur and iron, and possibly other elements, the entire complex forming the melanin. Sulphur and iron were considered as not indispensable constitutents of the melanin molecule.

While there is a lack of unanimity regarding the constancy of sulphur in the melanin molecule, so many workers have stated definitely that melanin contains sulphur that it seems apropos to delve more deeply into the ubiquity of sulphur in human cells. The recent attention given to the sulphydril compounds has necessitated research with the sulphur-containing amino-acids, the most important of which is cystine. Ropshaw [23] regarded melanogenesis as nature's microcolor reaction in which the cysteine-cystine complex acts as an indicator, revealing a phase of nuclear metabolism, viz., the cleavage of protamine and its diffusion into the cytoplasm. Cysteine, he stated, is present in the cytoplasm within the more complex body, the tripeptide glutathione, and protamine is present in nucleoprotein (chromatin). The cleavage caused by cell enzymes results in these two compounds being freed and brought in contact. In vitro, a black precipitate is formed when protamine and

20. Dyson, W.: J. Path. & Bact. **15**:298, 1910.
21. Piettre, M.: Compt. rend. Acad. d. sc. **153**:782, 1911.
22. Young, W. J.: Biochem. J. **8**:460, 1914.
23. Ropshaw, H. J.: Am. J. Physiol. **103**:535, 1933.

cystine are brought together. In time the color disappears. Possibly this may indicate the mechanism whereby melanin reverts to a colorless form. In the skin, incidentally, the sulphydryls are limited to the epithelial layer. Sulphur is also abundant in the appendages of the skin, such as the nails.

Hartwell [24] found that the more tyrosinase and tryptophan in the diet of rats the darker their coats became. Lignac,[25] on the other hand, showed that boiled tissues develop pigment under a quartz light and suggested that it was formed by a polymerization of quinidines. Also injections of pyrrole produced melanuria; therefore, melanin might be a simple cyclic complex of the pyrrole nucleus.

In most discussions on melanin, brown or blackish colors have been assumed, too uncritically it seems to me, to be melanin, or "a melanin," of "melanins." It is to be hoped that the term melanin can soon be used correctly only in the singular and with a definite idea as to a fundamental pigmented factor which, however, may have the ability to link itself with other substances, such as fat, lipoid, sulphur or metals, without altering its essential character.

Attempts at producing melanin in vitro have naturally had as their first aim the production of a black or brownish-black pigment. The aromatic compounds of the protein molecule, such as tyrosine, phenylalanine and tryptophan, readily condense with the elimination of water to form colored substances. Artificial melanin or melanoid substance can be formed by heating protein with hydrochloric acid. On decomposition with fused potassium hydroxide, it yields skatole, indole and pyrrole derivatives, derived from tyrosine and tryptophan. Bloch [26] has noted that the artificial melanin from tyrosine varies in its nitrogen content from 4.3 to 8.5 per cent. It seems probable that melanoid and melanin are formed from chromogen groups of the protein molecule through a process of condensation, elimination of water and the taking up of oxygen.

Brahn [27] derived a melanoidin from keratin and believed it very similar in composition to melanin to which he gave the structure 3, 4 *di*-hydroxyphenylcysteine.

In the ink sac of the cuttle fish and the meal worm and in plants producing Japanese lacquer there have been found oxidizing enzymes that can produce black pigment by their action on tyrosine solutions.

Neuberg,[28] with extracts of a melanosarcoma of the suprarenal gland, produced pigment from epinephrine and *B*-oxyphenylethylamine.

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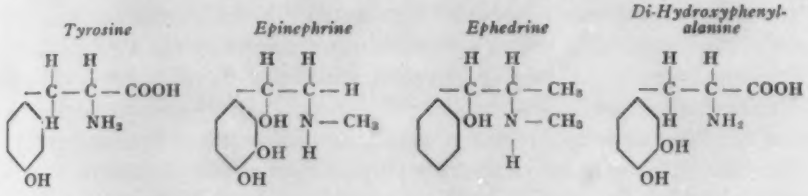
24. Hartwell, G. A.: Biochem. J. **17**:547, 1923.
25. Lignac, G. O. E.: Virchows Arch. f. path. Anat. **240**:383, 1923.
26. Bloch, B.: Am. J. M. Sc. **177**:609, 1929.
27. Brahn, B.: Virchows Arch. f. path. Anat. **227**:137, 1920.
28. Neuberg, C.: Ztschr. f. Krebsforsch. **8**:195, 1909.

but not from tyrosine. The ink sac of the squid contains an enzyme forming a pigment from epinephrine, also apparently through oxidation and condensation.

Winternitz [29] found in the uvea of the hog and the black subcutaneous tissue of the horse an enzyme causing darkening of tyrosine. These enzymes might hence be responsible for the production of melanin in animal tissues by causing oxidative changes in the chromogen groups of the protein molecule that are liberated by autolysis (Wells [30]).

It might be of advantage to indicate the structural relationships of tyrosine, epinephrine, ephedrine and *di*-hydroxyphenylalanine.



THE REACTION TO DI-HYDROXYPHENYLALANINE

Among the modern students of pigmentation the name of the late Bruno Bloch [31] of Zurich occupies a leading place. His studies have thrown much light on the origin of melanin and have been of great aid in the understanding of melanotic tumors, filling in certain gaps in the Soldan-Masson theory of the nervous origin of melanomas. In trying to account for the diffuse melanosis in Addison's disease, he reasoned that here was a condition in which the melanin deposits were associated with a lesion in an organ, the suprarenal gland, the main product of which is a pyrocatechin derivative, epinephrine.

He then used the cyclic amino-acid most closely related to epinephrine, *di*-hydroxyphenylalanine. Frozen sections of fresh skin were placed in 1 per cent *di*-hydroxyphenylalanine for from two to four hours at room temperature. In the cells which he regarded as those capable of forming melanin, the melanoblasts, the *di*-hydroxyphenylalanine was converted into a dark insoluble pigment, or *di*-hydroxyphenylalanine melanin. In other words, these cells contained an oxidase, or *di*-hydroxyphenylalanine oxidase. The cells did not develop melanin when exposed to pyrrole, tryptophan, tyrosine or epinephrine. The reaction appears to be definitely of a fermentative nature and is positive

29. Winternitz, R.: Arch. f. Dermat. u. Syph. **126**:252, 1918.

30. Wells, H. G.: (*a*) Chemical Pathology, Philadelphia, W. B. Saunders Company, 1925, p. 528; (*b*) p. 533.

31. Bloch, B., and Ryhiner, P.: Ztschr. f. d. ges. exper. Med. **5**:179, 1917.

only in the cytoplasm (an argument against the nuclear origin of melanin).

The reaction to *di*-hydroxyphenylalanine is an indicator of the presence of a pigment-forming oxidase, but *di*-hydroxyphenylalanine is not necessarily the mother substance of natural melanin. It indicates, however, that pyrocatechin derivatives may play a rôle as substrates in the formation of the natural pigment.

The only physiologic pyrocatechin derivative is epinephrine. Pyrocatechin derivatives are occasionally found in the urine of patients with general melanomatosis, melanemia and melanuria; hence Bloch thinks that it is logical to believe that the derivatives come from the mother substance of melanin, becoming free through the disintegration of tumor cells, finally entering the blood and being excreted by the kidneys. An important exception to the so-called specificity of *di*-hydroxyphenylalanine in indicating melanoblasts is the polymorphonuclear leukocyte the oxidase of which forms a black pigment with *di*-hydroxyphenylalanine. The enzyme concerned is probably a polyphenolase and is capable of oxidizing many phenols to colored products.

The reaction to *di*-hydroxyphenylalanine is of importance in the understanding of the chemistry of melanin. Its significance in cellular studies will not be discussed in extenso here. Bloch's main contribution basically was in showing that in the formation of melanin, ferment processes play an important rôle. Lemmel,[32] on the other hand, questioned the enzymatic nature of the reaction since he found the reaction to *di*-hydroxyphenylalanine more active in boiled than in fresh tissues, but it is difficult to reconcile this with the fact that *di*-hydroxyphenylalanine oxidase dissolves quickly in water and is dispersed (Laidlaw[33]).

Di-hydroxyphenylalanine, as can be seen from its molecular structure, is an amino-acid similar in some respects to epinephrine. It occurs in the Georgia velvet bean (Stizolobium deeringianum) and in the seeds of Vicia faba (Miller[34]). It reacts more or less like epinephrine but does not have the highly marked physiologic properties, although distinctly toxic. It is apparently the chromogen involved in certain brown and black animal pigmentations, such as butterfly wings (Vanessa antiopa, Hasebroek[35]).

Bloch and Schaaf[36] cited additional evidence for the specificity of *di*-hydroxyphenlalanine oxidase. The reaction to *di*-hydroxyphenylalanine of human skin is given only by the levorotatory *di*-hydroxy-

32. Lemmel, A.: Centralbl. f. allg. Path. u. path. Anat. **32**:89, 1921.
33. Laidlaw, G. F.: Am. J. Path. **8**:41, 1932.
34. Miller, E. R.: J. Biol. Chem. **44**:481, 1920.
35. Hasebroek, K., cited by Gortner, R. A.: Outlines of Chemistry, New York, John Wiley & Sons, Inc., 1928, p. 452.
36. Bloch, B., and Schaaf, F.: Klin. Wchnschr. **11**:10, 1932.

phenylalanine and not by the dextro form. Tyrosinase acts on both forms. The levorotatory compound is the natural form and is probably the propigment (Peck, Sobotka and Kahn [37]).

Spencer [38] has studied the chemical relationships of melanin, showing that protein derivatives like tyrosine, which contain the groups pyrrole, skatole or indole, are capable of being changed into melanin when acted on by certain enzymes which are found to occur frequently in plants and in the blood of insects and crustacea, and to their action the darkening of the blood of the animals on exposure to the air has been attributed (Alsberg [39]).

The dark color fungi assume when dying is dependent on an oxidizing ferment, for tyrosine, when mixed with an emulsion of fungi, turns black. The ferment obtained from the pupae of butterflies, when treated with tyrosine, forms a black pigment identical with melanin (McDonagh [40]). In 1887 Thormaehlen [41] discovered in the urine of patients suffering from melanotic cancer of the liver a substance which darkened on exposure to air, but was intensified by treatment with oxidizing agents. This discovery suggested that the mother substance of melanin might be a colorless chromogen, melanogen.

The subcutaneous injection of melanin into guinea-pigs and rabbits is followed by the appearance in the urine of a substance which turns dark brown after the urine has stood for several days (Helman [8]). The pigment is said to be changed by the liver to the colorless form and by oxidation in the urine again assumes its pigmented character. Oxidizing agents will hasten the reaction. Helman has laid down three criteria for the presence of melanogen in the urine: First, the addition of ferric chloride must cause the development of a black precipitate. Second, this precipitate must dissolve in a solution of sodium carbonate. Third, mineral acids must also precipitate a black or brownish-black powder. There may be confusion in cases of chronic phenol intoxication and extreme indicanuria (Feigl and Querner [42]).

Eppinger [43] noted that the feeding of tryptophan to patients with melanuria increases the excretion of melanin. He found that in patients with melanoma the power of the body to destroy the pyrrole ring is reduced, with the result that it undergoes reduction and methylation with sulphuric acid to form a sulphate of methyl-pyrrolidine-hydroxy-carbonic acid, $CH_3\text{-}C_5H_9N_2O_4$.

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37. Peck, S. M.; Sobotka, H., and Kahn, J.: Klin. Wchnschr. **11**:14, 1932.
38. Spencer, W. G.: Brit. M. J. **2**:907, 1923.
39. Alsberg, C. L.: J. M. Research **16**:117, 1907.
40. McDonagh, J. E. R.: Brit. J. Dermat. **22**:316, 1910.
41. Thormaehlen, J.: Virchows Arch. f. path. Anat. **108**:317, 1887.
42. Feigl, J., and Querner, E.: Deutsches Arch. f. klin. Med. **123**:107, 1917.
43. Eppinger, H.: Biochem. Ztschr. **28**:18, 1910.

Abderhalden [44] found a substance rich in tryptophan in the urine of a patient with melanuria. Primavera [45] noted in the urine of a patient with melanoma free tyrosine which fluctuated in amount with the pigment.

The pigment in Addison's disease has been assumed to be melanin. The circumstantial evidence is in favor of this assumption. Eiselt [46] isolated from the urine in Addison's disease a pigment rich in sulphur which he considered to be identical with melanogen. Jacobsen and Klinck,[47] utilizing the ability of melanin to reduce silver nitrate, found in the urine of two patients with Addison's disease renal casts containing a brownish and black pigment which was very argyrophilic and which they concluded was melanin. In the kidneys of patients with Addison's disease they also found much similar pigment in renal casts in situ and in the cells of the cortical and medullary tubules. The distribution of this pigment was the same as that in the kidneys of mice bearing large melanomas and in human melanomatosis.

Melanin, when injected parenterally, becomes decolorized by the reducing action of the tissues which blacken when oxidized. Quattini [48] injected pyrrole, indole and skatole subcutaneously into rabbits and found that in from nine to eighteen days the skin became darkened, and there was increased growth of pigmented hair. These observations suggest that a mother substance belonging to the indole group may be acted on by an enzyme produced in the epidermal cells, with the production of a melanin pigment (Dawson [49]). When melanin from melanotic tumors is treated with strong alkali, it yields indole and skatole and various volatile fatty acids, and under dry heat melanin gives off pyrrole.

Melanosis coli or melanotic pigmentation of the colon is of great interest in the study of the formation of melanin. Beneath an intact epithelial covering the stroma of the mucosa of colon, and often too of the appendix, is crowded with cells containing a brownish-black pigment which in the gross gives a grayish-black color to the lining of the intestine. The pigment looks like melanin and has affinity for silver salts. The pigment-bearing cells are negative to *di*-hydroxyphenylalanine, indicating that they do not actually form the pigment and hence must be phagocytes which have engulfed it. The condition apparently occurs only in very constipated people or when there is a chronic intestinal obstruction of mechanical origin.

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44. Abderhalden, E.: Ztschr. f. physiol. Chem. **78**:159, 1912.
45. Primavera, cited by Wells, H. G.: Chemical Pathology, Philadelphia, W. B. Saunders Company, 1925, p. 531.
46. Eiselt, R.: Ztschr. f. klin. Med. **69**:393, 1910.
47. Jacobsen, V. C., and Klinck, G. H., Jr.: Arch. Path. **17**:141, 1934.
48. Quattini, M., cited by Spencer.[38]
49. Dawson, J. W.: Edinburgh M. J. **33**:657, 1925.

Recently, in discussing the mobilization and excretion of melanin, Jacobsen and Klinck [47] suggested that melanosis coli is possibly evidence that the intestinal tract is one of the main routes for the escape of melanin after its discharge from effete body cells normally containing it. They had shown also that the kidneys are another important channel for the escape of surplus melanin. In melanosis coli there are complicating factors which in view of what is known of the composition of the melanin molecule make it necessary to consider the possibility of synthesis of melanin in the intestinal canal, the amino-acids and tyrosinase probably being present, and the whole being more of an in vitro than an in vivo process.

Ludwig Pick,[50] however, expressed the belief that the aromatic protein degradation products are absorbed from the large intestine and converted into melanin within the connective tissue cells by a ferment resembling tyrosinase. The melanin in melanosis coli varies somewhat in its reactions, at times reacting slowly to silver nitrate and being bleached by hydrogen dioxide with more difficulty than is the pigment of the skin (Lignac [25]).

An interesting but rare disease which throws some light on the chemical relationships of melanin is ochronosis. In 1866 Virchow first described it with its pigmentation of the cartilages and ascribed the pigment to hemoglobin derivatives. Von Hansemann [51] noted melanuria with the condition, and Hecker and Wolf [52] confirmed this observation. Albrecht [53] suggested a relationship to alkaptonuria, having found homogentisic acid in the urine. In 1906 Ludwig Pick [54] studied the condition and summarized the matter as follows:

> Ochronosis is a definite form of melanotic pigmentation, the pigment of ochronosis being in most cases closely related to melanin. The pigment or its chromogen, circulating freely in the blood, is imbibed not only by cartilage, but also by loose connective tissue, voluntary and involuntary muscle cells and epithelial cells, without any obvious decrease in vitality of these cells. Degenerated tissues show the greatest amount of pigmentation. The diffuse pigment may become granular after a time. It is iron-free, but under certain circumstances may contain fat. The melanin arises from the aromatic nucleus of the protein molecule (tyrosine, phenylalanine), and the related hydroxylized products, under the influence of tyrosinase.

In some cases the constant absorption of minute quantities of phenol from surgical dressings has apparently caused the condition. Besides this formation of pigment from such exogenous aromatic substances,

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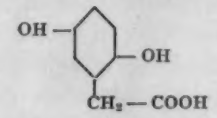
50. Pick, L.: Berl. klin. Wchnschr. **43**:478, 1906.
51. von Hansemann, D.: Berl. klin. Wchnschr. **29**:660, 1892.
52. Hecker, A., and Wolf, H., cited by Wells, H. G.: Chemical Pathology, Philadelphia, W. B. Saunders Company, 1925, p. 532.
53. Albrecht, H.: Ztschr. f. Heilk. **23**:366, 1902.
54. Pick, L.: Berl. klin. Wchnschr. **48**:840, 1911.

it is probable that in alkaptonuria the endogenous aromatic substance, homogentisic acid, may be converted into pigment by tyrosinase. In many cases of ochronosis the pigment or a precursor may be excreted in the urine, which then undergoes spontaneous darkening when exposed to the air. The kidneys may also become pigmented, and granular masses of pigment may be present in the renal tubules. The evidence, as Wells [30b] stated, is quite conclusive of the origin of ochronotic pigment from aromatic radicals, whether these radicals are converted into true melanin or not. The structure of homogentisic acid is:



A type of ochronosis which affects the bones rather than the cartilages in the lower animals also occurs. Schmey [55] has called this condition osteohemochromatosis, and others have called it osteohematophyria. If it is comparable with a pigmentation of bones which occurs in fish, the pigment is not of blood origin but is true melanin in all probability. Harper [56] observed extensive melanism in the skeleton of Raia maculata, every portion of the skeleton containing a deposit of melanin pigment. The deposit was heaviest in the main cartilages. The pigment was extracted with methyl alcohol acidified with hydrochloric acid. This solution after filtration showed no absorption bands, which excluded all compounds of the porphyrin class and, in particular, uroporphyrin. It was bleached by hydrogen dioxide or nitric acid, indicating that the pigment probably belongs to the melanin type. Gross [57] has produced ochronosis experimentally by the administration of homogentisic acid, but numerous cases are on record of alkaptonuria without ochronosis. Some profound metabolic disorder involving the catabolism of tyrosine and phenylalanine seems at fault, and usually it is of hereditary nature. The presence of homogentisic acid in the urine should not be taken necessarily as an indication of a pathologic condition, since an increased consumption of proteins containing tyrosine and phenylalanine will increase the amount of homogentisic acid which is excreted.

In spite of much contradictory research on the structure of the melanin molecule, there appears to be general agreement on certain basic facts. When tyrosinase acts on tyrosine, a series of changes in color occurs, most rapidly at p_H 6 and more slowly between p_H 6 and 8.

55. Schmey, M.: Frankfurt. Ztschr. f. Path. **12**:218, 1913.
56. Harper, W. F.: J. Path. & Bact. **35**:865, 1932.
57. Gross, O.: Deutsches Arch. f. klin. Med. **128**:249, 1919.

At first a red color develops which passes into a brown or black. The red color, according to Laurens,[58] becomes decolorized, forming an easily oxidizable substance. The red color is the primary result of the action of the tyrosinase, the subsequent changes being purely oxidations. It is probable that the first product of the action of tyrosinase on tyrosine is the formation of *di*-hydroxyphenylalanine. On oxidation this becomes the corresponding quinone, which in turn is converted into 5:6 *di*-hydroxy-*di*-hydroindol-2-carboxylic acid. This by oxidation becomes the corresponding quinone, which is probably the red substance, the first visible product of the enzyme action. When the red substance is decolorized it gives rise to 5:6 *di*-hydroxyindol or its 2-carboxylic acid, both probably easily oxidized in the air and one of which must be the colorless melanogen.

Since the only physiologic pyrocatechin derivative is epinephrine, and since pyrocatechin derivatives play their most conspicuous rôle in melanomatosis, the coincidence carries weight as indicating that pyrocatechin derivatives come from the mother substances of melanin, which are formed in great amount, are freed through break-up of tumor cells, enter the blood and are eliminated through the kidneys and probably also through the intestinal tract.

Melanin has some of the characteristics of a true protein or colloid and is classed by Gortner as a conjugated protein, subgroup chromoprotein or melanoprotein. The recent studies of Adant seem to indicate that melanin has also one of the biologic properties of proteins; i. e., it is antigenic. A knowledge of this fact opens a field of research which may result in important discoveries concerning normal and pathologic pigmentation. An appreciation of the normal variations in the threshold of secretion and excretion of melanin in conjunction with an accurate estimate of its antigenic potency may lead to the development of a serologic method for the determination of familial and racial characters based on potential or actual melanogenesis.

58. Laurens, H. L.: Physiological Effects of Radiant Energy, New York, The Chemical Catalog Company, Inc., 1933, p. 134.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—According to *Science*, G. P. Wright has been appointed to the Sir William Dunn chair of pathology tenable at Guy's Hospital Medical School. Since 1931 he has been assistant lecturer in morbid anatomy and curator of the Museum at University College Hospital Medical School and also pathologist of the hospital.

Bradley M. Patten, associate professor of histology and embryology in Western Reserve University has resigned to become assistant director for medical sciences for Rockefeller Foundation.

Albin Haberda, professor of legal medicine in the University of Vienna and author of a widely used textbook of legal medicine, has died at the age of 66, after a long illness.

Society News.—At a conference held in Washington on Feb. 5 and 6, 1934, under the auspices of the National Research Council there was organized the American Academy of Tropical Medicine with Theobald Smith as president, Charles F. Craig as vice-president, W. W. Cort as treasurer, E. B. McKinley as secretary, and a council of five.

George B. Hassin has been elected president, and Armando Ferraro secretary, of the American Association of Neuropathologists.

The German Pathological Society will hold its next meeting in Rostock May 23 to 25, 1934.

Bequests.—According to *Science News-Letter*, the University of Wisconsin has received a bequest from the late Miss Jean Bowman to found a cancer clinic and research center.

Grants-in-Aid.—The Committee on Grants-in-Aid of the National Research Council announces that it has received a new grant for the year 1934. Formal blanks for use in applying for a grant will be furnished on request to the committee, 2101 Constitution Avenue, Washington, D. C. The Committee on Scientific Research of the American Medical Association has been given a new appropriation by the trustees of the Association. Application blanks will be sent on request to 535 North Dearborn Street, Chicago.

Obituaries

MAJOR PAUL EDGAR McNABB, M.C., U.S.A.

1887–1934

Major Paul Edgar McNabb died on Feb. 24, 1934, from a rapidly progressive hypertensive disease and cerebral hemorrhage. He was born in Sevier County, Tenn., on May 23, 1887, and at the age of 5 years moved with his parents to Knoxville, Tenn., where he spent his

MAJOR PAUL EDGAR McNABB, M.C., U.S.A.
1887–1934

boyhood. He graduated in medicine at the University of Pennsylvania in 1912, and then practiced medicine for several years with his father, Dr. Charles P. McNabb, a distinguished physician in Knoxville.

Major McNabb was an honor graduate of the Army Medical School in 1917, and served in France during the World War. He graduated from the advanced course, Army Medical School, in 1921, and was assigned to the laboratory service of the medical department, where he served with distinction in many difficult assignments, including the

Second Corps Area Laboratory, New York; Board of Health Laboratory, Ancon, Canal Zone; Walter Reed General Hospital, and the Army Medical School. During this service he came to be regarded as one of the outstanding pathologists in the Army Medical Corps. He was curator of the Army Medical Museum from February, 1931, to January, 1933, and was president of the U. S. Army Medical Department Research Board in Manila, P. I., at the time he was stricken with the illness which forced him to return to the United States.

Always interested in research, he published articles on postmeasles pneumonia, quinine prophylaxis in army troops in the Canal Zone, coronary sclerosis in angina pectoris and the presence and significance of albuminuria in the personnel of a citizens' military training camp. In collaboration with others, he published articles on hemochromatosis and congenital heart block.

Major McNabb was a fellow of the American Medical Association and of the American College of Physicians and was a member of the American Association of Pathologists and Bacteriologists, the International Association of Medical Museums and the National Board of Medical Examiners representing the U. S. Army Medical Corps. He was also an honorary life member of the Knox County Medical Society.

An indefatigable worker for the interests of the laboratory service, Major McNabb was keenly interested in the welfare and advancement of its personnel, especially of the junior members of that service. Possessed with professional ability, charming personality and more than ordinary tact, he quickly obtained the friendship and respect of those who were privileged to be associated with him. His untimely death during the years of greatest productivity is a distinct loss to the Medical Corps.

In 1913, he married Miss Therese Franz of Knoxville, who with their daughter, Jane Gordon McNabb, aged 17, survives him.

V. H. CORNELL.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

COMPLETE HEART-BLOCK IN HYPERTHYROIDISM FOLLOWING ACUTE INFECTIONS. AUSTIN C. DAVIS and HARRY L. SMITH, Am. Heart J. **9**:81, 1933.

Six cases of exophthalmic goiter in which complete auriculoventricular dissociation occurred were observed. Three of the patients had had acute tonsillitis shortly preceding the arrhythmia, and one patient had had scarlet fever. In the one fatal case, there was an inflammatory lesion in the region of the bundle of His, in which were gram-positive cocci. In the two cases in which the heart block was not associated with acute infection, the block may have been precipitated by the effect of digitalis on a previously injured auriculoventricular bundle. In those cases in which acute infection was present, the evidence indicates that the hyperthyroidism predisposed the patient to the development of acute infection and also to secondary myocardial involvement.

FROM AUTHORS' SUMMARY.

LYMPHATIC LEUKEMIA FOLLOWING BENZOL POISONING. ERNEST H. FALCONER, Am. J. M. Sc. **186**:353, 1933.

A case of benzene poisoning is presented in which the patient recovered for a time, showing a lower level of blood cell production, which gradually passed into true lymphatic leukemia (verified at autopsy) with a fatal termination. Instances are drawn from the literature suggesting that benzene, like other agents destructive of hemopoietic tissue, may upset the balance of an orderly production and destruction of blood cells, bringing about a tissue environment favorable to the production of lymphatic leukemia. Attention is directed to the direct division of the nucleus in many lymphatic cells in the blood stream and in lymphatic tissue cells in lymph nodes and bone marrow.

AUTHOR'S SUMMARY.

POSTERIOR PITUITARY ACTIVITY FROM AN ANATOMICAL STANDPOINT. HARVEY CUSHING, Am. J. Path. **9**:539, 1933.

In all the recent attention to the pituitary body and its functions, the posterior lobe has been much neglected because of the greater temporary interest in the hormones of the pars anterior and the newly discovered activities of the interbrain. It nevertheless has an active principle or principles capable among other properties (1) of raising the blood pressure, (2) of contracting smooth muscle, (3) of causing hyperglycemia, (4) of expanding cutaneous melanophores and (5) of diminishing renal secretion. Its secretory product is unmistakably derived from the investing pars intermedia, the cells of which become basophilic when ripened. Doubtless, under nervous impulses from hypothalamic nuclei, these cells, by a form of holocrine secretion, are cast off, invade the pars nervosa and become transformed into "hyaline bodies," which, as Herring first showed in lower animals, make their way through the loose neural spaces of the pars nervosa in the direction of the infundibular cavity. They may, in favorable preparations from the cat and dog, even be seen to extrude themselves into the ventricle between the ependymal cells. In certain conditions of disease characterized notably by hypertension, but also by other symptoms suggestive of known physiologic effects of posterior lobe extracts, the normal cellular activity of the pars intermedia becomes greatly exaggerated. This is shown by a marked hyperplasia of the basophilic elements that penetrate far into the lobe. Under these circumstances, not only is the visible secretory product (hyalin) greatly increased in amount over what is customarily seen in supposedly normal glands, but the cuticular ependyma of the lower third ventricle

has a highly broken up appearance. An extreme degree of cellular hyperactivation of the posterior lobe was first observed in the pituitary body in a fatal case of what is known as pituitary basophilism associated with a basophilic adenoma of the pars distalis. Since then, a similar condition has also been found in cases of eclampsia and of so-called essential hypertension. In both of these states, there has been found in the blood stream a substance indistinguishable in its effects from posterior lobe extract, but not detectable in the blood under normal conditions. The conclusion is drawn that the infiltrative basophilia of the pars nervosa is an expression of functional hyperactivation of the posterior lobe and may be taken to represent the pathologic basis of these hypertensive disorders. What particular form of neurohumoral stimulus primarily incites the neurohypophyseal basophilia remains to be determined.

AUTHOR'S SUMMARY.

PHOSPHORUS AND ALCOHOLIC CIRRHOSIS. F. B. MALLORY, Am. J. Path. **9**:557, 1933.

Very chronic poisoning with minute daily doses of yellow phosphorus produces, in five months or more, cirrhosis of the liver in rabbits and in guinea-pigs. The hepatic cells undergo degenerative changes similar to those found in alcoholic cirrhosis. They contain granules and hyaline networks (reticula) that are acidophilic and give the reaction for phosphates by the Macallum and Fiske-Subbarow tests. By a special method, it is possible to demonstrate free phosphorus when cast iron and tin plate are dissolved in dilute hydrochloric acid. Phosphorus may get into alcohol and liquors through acid erosion of iron and tin plate vessels and containers and thus contaminate them and be the cause of alcoholic cirrhosis.

AUTHOR'S SUMMARY.

ERYTHROLEUCOBLASTOSIS IN THE NEWBORN. R. C. WANSTROM, Am. J. Path. **9**:623, 1933.

A case of fetal erythroleukoblastosis with ascites is reported, in which microscopic study revealed certain unusual features. In addition to marked hematopoiesis in the liver, spleen, kidneys, bone marrow, lymph nodes, thymus, diaphragm, pharynx, small intestine and pancreas, similar foci were found in the chorionic villi. In the lesser bile ducts and in some renal tubules numerous giant cells were found, apparently resulting from alterative changes in damaged epithelium. The generic relationship and probable intrinsic constitutional etiology of congenital hydrops, icterus gravis and fetal erythroleukoblastosis of the new-born are pointed out and suggestions made as to future investigations along these lines.

AUTHOR'S SUMMARY.

EFFECT OF TESTICULAR EXTRACT ON EXPERIMENTAL TUBERCULOSIS IN RABBITS. T. T. WALKER and D. C. HOFFMAN, Am. J. Path. **9**:651, 1933.

When tubercle bacilli together with testicular extract are injected into the skin of rabbits, marked enhancement of the resulting lesions occurs. This enhancement takes place equally well with the human, bovine and avian strains. The practical application of this phenomenon in laboratory diagnosis is worthy of consideration.

AUTHORS' CONCLUSIONS.

BLOOD CHOLESTEROL IN EXPERIMENTAL THYROID DISEASE. J. J. WESTRA and M. M. KUNDE, Am. J. Physiol. **103**:1, 1933.

Artificially cretinized rabbits showed a cholesterol content of the blood greater than normal; after the feeding of desiccated thyroid, this was reduced—a phenomenon which does not always occur after a similar procedure in normal rabbits. No correlation could be established between these changes and the alterations of red cells and hemoglobin.

H. E. EGGERS.

EXPERIMENTAL ALOPECIA. B. BARKER BEESON and W. J. PICKETT, Arch. Dermat. & Syph. **28**:53, 1933.

The sympathetic ganglion of the second cervical nerve was removed bilaterally in twenty-one cats. Seventeen of them survived, and six of the survivors showed postoperative alopecia, which appeared from the fourth to the fortieth day. The loss of hair attained its maximum in several weeks and then remained stationary for one or two weeks, after which the hair reappeared, becoming normal in from one to two and one-half months. Histologically atrophy and disappearance of hairs, hair follicles and sebaceous glands were noted. The subdermal connective tissue was thickened. These histologic changes are similar to those found in alopecia areata in human beings.

S. W. BECKER.

EFFECT ON THE REFLEXES OF THE CAROTID SINUS OF RAISING THE INTRACRANIAL PRESSURE. C. M. GUERNSEY, S. A. WEISMAN and F. H. SCOTT, Arch. Int. Med. **52**:306, 1933.

The rise of arterial pressure following an increase of intracranial pressure is, in dogs and rabbits, of central origin, as Cushing thought, and does not start in the carotid sinus. In the authors' experiments, the carotid sinus was always found to act as a depressor zone, tending to keep the pressure down even when the nerve centers needed blood. Following denervation of the sinus, increased intracranial pressure caused a more prompt and greater rise of arterial pressure. The differences before and after denervation were not great, and this was true in rabbits in which the influences of the aortic depressors also were removed.

AUTHORS' SUMMARY.

GALL BLADDER EPITHELIUM AS AN OSTEOGENIC STIMULUS AND THE PHYSIOLOGICAL DIFFERENTIATION OF CONNECTIVE TISSUE. C. B. HUGGINS and J. F. SAMMET, J. Exper. Med. **58**:393, 1933.

Evidence is presented that the proliferating epithelium of the gallbladder in the dog and guinea-pig is capable of stimulating the formation of bone in certain connective tissues such as the abdominal wall. Other areas of connective tissue such as the subepithelial connective tissue of the glallbladder and the urinary bladder do not share in this reaction and resist the osteogenic stimulus of the epithelium. In these circumstances, this formation of bone is biphasic. A difference between connective tissues morphologically identical can be proved physiologically by their response to the osteogenic stimulus of appropriate epithelia. Microliths of calcium carbonate occurred in the mucus of the occluded gallbladder in which there was transplanted connective tissue forming part of the wall.

AUTHORS' SUMMARY.

THE RACES THAT CONSTITUTE THE GROUP OF COMMON FIBROBLASTS. R. C. PARKER, J. Exper. Med. **58**:401, 1933.

Paces of fibroblasts that are functionally distinct have been isolated from the various tissues and organs of a single chick embryo. Functionally distinct races of fibroblasts have also been isolated from corresponding parts of embryos of different ages. Under the conditions of the experiments, and for the particular races of fibroblasts that have been studied, it has not been possible to demonstrate a gradual decrease, or a gradual increase, in the rate of multiplication of fibroblasts obtained from corresponding parts of embryos of gradually increasing ages. Experiments made on strains of fibroblasts derived from the mesonephron and metanephron of a 16 day chick embryo have indicated that the rate of multiplication of these cells in a given medium reflects the physiologic state, at the moment of isolation, of the particular part of the embryo from which they are obtained. The rate of multiplication of a given race of fibroblasts in a particular medium does not serve, necessarily, as an index of the age of the individual from which

the race is derived. The functional differences that distinguish the various races of fibroblasts are permanent; they are retained indefinitely by the cells from passage to passage, despite such attempts as have thus far been made to change them.

AUTHORS' SUMMARY.

SYNERGIC HYPOPHYSEAL FACTOR AND PROLAN LIKE SUBSTANCES. H. M. EVANS, M. E. SIMPSON and P. R. AUSTIN, J. Exper. Med. **58**:545 and 561, 1933.

The hypophyseal substance—the synergic factor—which gives increased gonadotropic effects when combined with prolan, has been shown itself to possess a definite though slight gonadotropic activity. It produces transitory follicular enlargement within from twenty-four to forty-eight hours, which, however, regresses at once on cessation of treatment, so that by the end of the fourth day the ovary again approximates its infantile weight. The synergic principle indeed provokes only moderate ovarian development when administered in one hundred times the dose necessary to demonstrate the phenomenon of activation. By means of iso-electric precipitation or by means of tryptic and ereptic digestion, fractions containing the synergic principle were freed of many contaminants, in particular of the antagonistic factor. The synergic principle has been shown to be unaffected by digestion with trypsin or with trypsin followed by a short digestion with erepsin, but the action of pepsin inactivated the material. A preparation of the active substance has been obtained which is potent in a total dose of 27 micrograms.

The phenomenon of synergism has made possible the recognition of substances which the authors have called "prolan-like" in a wide variety of conditions. Indeed, it has been possible to demonstrate a prolan-like material in the urine of normal men. The method has shown a wide distribution of prolan-like substances in serums and urines of nonprimates without demonstrating, however, a significant difference in the reactions secured from pregnant, as contrasted with nonpregnant, states, or in males as contrasted with females. The phenomenon of synergism with the prolan of pregnancy can also be shown in the increased development of the seminal vesicles of immature male rats. However, such males cannot be advantageously employed in the detection of prolan-like gonadotropic substances occurring in the blood and urine in conditions other than pregnancy, for the prolan-like substances usually do not effect appreciable development of the seminal vesicles.

AUTHORS' SUMMARIES.

CONCENTRATION OF THE GONADOTROPIC HORMONE IN PREGNANT MARE'S SERUM. H. M. EVANS, E. L. GUSTUS and M. E. SIMPSON, J. Exper. Med. **58**:569, 1933.

The gonadotropic hormone of the blood of the pregnant mare has been greatly concentrated by adsorption on active aluminum hydroxide followed by elution. The preparations so obtained gave demonstrable gonadotropic effects within one hundred hours in 21 day old female mice following three subcutaneous injections of 0.001 mg. in 1 cc. of physiologic solution of sodium chloride. As is well known, other gonadotropic substances do not cause conspicuous development of the male gonads, but injections of comparatively large doses of these preparations into immature male rats caused marked development of the testes, which in ten days were trebled in weight. An astonishing increase in the weight of the seminal vesicles resulted, for these organs were approximately seventy-five times heavier than in controls.

AUTHORS' SUMMARY.

CULTIVATION OF MONOCYTES IN FLUID MEDIUM. L. E. BAKER, J. Exper. Med. **58**:575, 1933.

Monocytes from blood and from spleen have been cultivated in fluid medium in Carrel flasks for over two months. Diluted serum supplied all the essential

nutritive substances. Cultivation in fluid was made possible by adjusting the initial p_H of the fluid to 7.4, and not allowing it to fall below 7 or 6.8. The cells remained in good condition when the p_H was adjusted with lactic acid, hydrochloric acid or carbon dioxide. Adjustment with carbon dioxide was found to be more convenient and also more practical, since it does not destroy the buffer action of the medium. After two months of cultivation, the monocytes were in excellent condition and still proliferated actively. They gave every indication that indefinite multiplication could be maintained under the conditions of these experiments. It is hoped that this method of cultivation, with some modifications, will prove useful in studying the metabolism of these cells.

AUTHOR'S SUMMARY.

PRODUCTION IN DOGS OF CHRONIC BLACK TONGUE WITH ANEMIA. C. P. RHOADS and D. K. MILLER, J. Exper. Med. **58**:585, 1933.

By the feeding of a particular diet, apparently lacking a substance closely associated with vitamin B_2 G, a chronic disease may be produced irregularly in dogs. The disease is characterized by atrophic glossitis, diarrhea, loss of weight and anemia. The disease can be prevented and relieved by materials rich in vitamin B_2 G.

AUTHORS' SUMMARY AND CONCLUSIONS.

METHODS AND EFFECTS OF INCREASING THE URINARY CONSTITUENTS IN THE BODY. F. W. HARTMAN, J. Exper. Med. **58**:649, 1933.

Five methods for the production of slow, continued reabsorption of urine in the experimental animal are presented. The effects of this prolonged reabsorption of urinary constituents on the blood chemistry and tissues are shown with special reference to the kidney. Large and rapid increase of urinary constituents in the body is definitely destructive to the kidney. The degenerative changes found in the experimental animal in the protected pole of the kidney showing nephritis produced by roentgen rays and in man in the unscarred portions of the kidney showing chronic nephritis account for the progressive nature and final renal insufficiency of these conditions and are best ascribed to the high levels of excretory products in the body.

AUTHOR'S CONCLUSIONS.

THE RELATIVE RESPONSE TO BLOOD GAINS AND BLOOD LOSSES. M. DICK, J. Exper. Med. **58**:707, 1933.

The effects of gradually increasing or diminishing the amount of circulating hemoglobin have been studied in rabbits. Contrary to expectation, it was found that when the pigment was increased by the injection of a small quantity of compatible blood every day during a number of weeks, the erythropoietic tissue did not lessen its activities. The percentage of hemoglobin mounted gradually yet considerably, even when as little as one hundredth of the amount of blood initially possessed by the animal was injected each day; and the figure it finally attained must in some instances, at least, have been expressive of a superabundance. To this superabundance the animal itself evidently contributed through its persisting erythropoietic activity. The results were very different when rabbits were bled daily, the same small amount of blood being lost that was injected into their fellows. The marrow became abnormally active, and this activity continued undiminished throughout the long period of the bleedings. The organism is evidently far more susceptible to loss than to gain of blood, a fact which is scarcely surprising when one considers that throughout its differentiation, it has had to cope with exigencies of the first sort only. Rabbits in which the hemoglobin is gradually increased by the injection of strange blood become so accustomed to the abundance of pigment that even a slight decrease in amount causes the erythropoietic tissue to become abnormally active to maintain the new status quo. Good reasons exist for referring the habituation thus manifested to readjustments in the functioning of the physiologic mechanisms which mediate between the demand for oxygen and the erythro-

poietic response. Too little recognition has been given to the rôle of these mechanisms in such a relation. No evidence was obtained of any effective readjustment to protect the erythropoietic tissue from the stimulus of daily small loss of blood.

AUTHOR'S SUMMARY.

FAILURE OF ADMINISTRATION OF IRON TO CAUSE HAEMOCHROMATOSIS. C. POLSON, Brit. J. Exper. Path. **14**:73, 1933.

The iron content of the liver of thirty-six adult rabbits was maintained at a high level for from one to four years. The terminal level in ten rabbits under experiment from three to four years ranged from 3.42 to 7.67, and averaged 5.19 per cent by dry weight. Cirrhosis of the liver and pancreatic damage were absent from all. It is therefore shown that excess of iron in the body over long periods caused neither cirrhosis of the liver nor hemochromatosis in rabbits, and it is unlikely that excess of iron is responsible for the hepatic and pancreatic lesions of human hemochromatosis.

AUTHOR'S SUMMARY.

THE CORTICAL LIPOID OF THE MOUSE SUPRARENAL AFTER UNILATERAL SUPRARENALECTOMY. RAYMOND WHITEHEAD, Brit. J. Exper. Path. **14**:149, 1933.

The relative amounts of sudanophil cortical lipoid in both suprarenals of 137 unilaterally suprarenalectomized male white mice have been estimated. Right suprarenals removed at intervals of from three to fifty-six days after removal of the corresponding left glands showed a decrease in the amount of lipoid. The proportion of right glands with decreased lipoid rose to a maximum of 68 per cent at fourteen days after removal of the left glands, and gradually fell to 17 per cent at fifty-six days. Some right glands showed increased lipoid from twenty-one to fifty-six days after removal of the left glands. Control laparotomies on twenty-four mice demonstrated that the lipoid changes were not due to the surgical technic. The results indicate that the amount of lipoid in depleted right glands in time attains or exceeds its original value. Histologic types of lipoid change are described and illustrated. Lipoid changes as sources of error are discussed. No evidence of cortical hypertrophy in twenty-nine right glands removed fifty-six days after removal of the left was obtained.

AUTHOR'S SUMMARY.

RENAL LESIONS IN HYPERVITAMINOSIS D. J. GOUGH, J. B. DUGUID and D. R. DAVIES, Brit. J. Exper. Path. **14**:137, 1933.

The occurrence of calcification and parenchymatous degeneration ("nephrosis") in the rat's kidney in hypervitaminosis D was investigated. The two lesions, which frequently coexist, were found to be largely independent of one another. Calcification went parallel with a high renal excretion of calcium. Nephrosis could not be clearly correlated with any of the urinary disturbances recorded, but seemed to be in a large measure dependent on the administration of sodium biphosphate, U. S. P., with overdoses of vitamin D. The effects of large doses of the acid and alkaline orthophosphates, both with and without hypervitaminosis D (20,000 M.R.C. units of vitamin D substance daily), were compared. The urinary excretion of calcium and the deposition of calcium in the kidneys were found to be greatest when the alkaline phosphate was given, and especially when the vitamin D substance was added. The urinary excretion of phosphorus was greatest when the acid phosphate was given. The vitamin D substance had the effect of lowering it, both when the acid and when the alkaline salt was given. The amount of phosphorus found post mortem in the kidney was greatest when the vitamin D substance had been given with the acid diet, and it was in these circumstances that the most severe nephrosis developed. The large accumulation of phosphorus in the kidney in the nephrotic rats was thought to be a mark of retention of phosphates

caused by the renal disease. The urinary excretions of calcium and phosphorus appeared to show a reciprocal relationship to one another, a relative elevation of the one being accompanied by a corresponding lowering of the other.

AUTHORS' SUMMARY.

EXPERIMENTAL HYPERTENSION AND THE ARTERIAL LESIONS IN THE RABBIT. M. KREMER, SAMSON WRIGHT and R. W. SCARFF, Brit. J. Exper. Path. **14**:281, 1933.

The aortic and sinus nerves (buffer nerves) were extirpated in rabbits which then were allowed to survive for long periods. The changes in the blood pressure were recorded by the carotid loop method. Following unilateral denervation, there is an elevation of the blood pressure from the normal level (about 95 mm.) to about 115 mm. Following bilateral denervation, there is a persistent marked hypertension, the average blood pressure being 160 mm. Degenerative changes in the aorta and fibrosis of the heart may take place in a high proportion of cases following bilateral denervation. These changes are not observed in control animals or after unilateral operation. The mode of production of the lesions is uncertain, but they are not the invariable accompaniment of hypertension.

AUTHORS' SUMMARY.

Pathologic Anatomy

EXFOLIATIVE DERMATOSIS AND MALIGNANT ERYTHRODERMIA. HAMILTON MONTGOMERY, Arch. Dermat. & Syph. **27**:253, 1933.

Seventy-three cases of exfoliative dermatitis and sixteen cases of generalized erythrodermia were studied. Exfoliative dermatitis following psoriasis presents a histologic picture similar to that of psoriasis, differing only in the more marked intercellular edema. Exfoliative dermatitis following eczema presents a varying degree of spongiosis. Exfoliative dermatitis due to arsenical poisoning presents a picture often indistinguishable from that of the same condition following eczema. In exfoliative dermatitis as a part of the picture of lymphoblastoma (Hodgkin's disease, leukemia, lymphosarcoma, mycosis fungoides) the amount of infiltrate in the cutis is larger, and the character differs, depending on the type of case. In mycosis fungoides the infiltration is limited to the upper portions of the dermis and is composed of a multiplicity of cell types, with pyknosis and karyorrhexis of the individual cells and a tendency toward clumping of many of the cells. The cells consist of eosinophils and polymorphonuclear leukocytes, fibroblasts, cells of the vessel walls and cells of the lymphocytic series. In Hodgkin's disease of the skin Dorothy Reed cells are found. In leukemia cutis there is dense infiltration with cells of the lymphocytic series or of the myelocytic series. Lymphosarcoma differs from lymphatic leukemia only in the numerous mitotic figures. In more than half of the cases of lymphoblastoma it is impossible definitely to specify which type of lymphoblastoma will eventually develop. It still seems advisable to attempt classification of the lymphoblastoma group as separate but related entities.

S. W. BECKER.

MYCOSIS FUNGOIDES. OLIVER S. ORMSBY and CLARK W. FINNERUD, Arch. Dermat. & Syph. **27**:631, 1933.

The case of a woman, aged 32, who had the prefungoid stage of mycosis fungoides which terminated fatally is reported. Sections from various regions of the skin showed the characteristic polymorphous cellular infiltrate simulating a granuloma. A periaortic abdominal lymph gland showed a delicate fibrillar network containing lymphocytes, eosinophilic polymorphonuclear leukocytes, a few lymph channels and a very few larger cells with a large nucleus, sparse chromatin and an abundance of eosinophilic cytoplasm. The spleen showed lobule-like proliferation about the central arteries. The authors believe that the changes in the spleen

and probably those in the lymph glands were similar and analogous to those in the skin, the pathologic cells in these internal structures representing a hyperplasia apparently derived from the lymphoid cells and the reticulum. They believe that the histologic changes are inflammatory or progressive and not due to an infiltration of neoplastic tissue. They also believe that the preponderance of clinical and histologic evidence demands the continued recognition of mycosis fungoides as a disease sui generis.

S. W. BECKER.

PULMONARY EMBOLISM FROM ARSENICALS INJECTED INTRAVENOUSLY. GEORGE C. SHIVERS, Arch. Dermat. & Syph. **27**:901, 1933.

A case is reported in which death resulted from a pulmonary embolism following the intravenous injection of 0.9 Gm. of neoarsphenamine as an initial dose. The author found that a low p_H caused precipitation of arsphenamine, neoarsphenamine, sulpharsphenamine and mersalyl in serum in vitro, and caused embolism when injected into rabbits. He found a surprisingly high number of low p_H values in neoarsphenamine obtained in the open market. The author advises testing neoarsphenamine by means of bromthymol blue, which has a sharp end-point at p_H 7 when combined with neoarsphenamine.

S. W. BECKER.

PRODUCTIVE-CICATRICIAL SYPHILITIC DISEASE OF THE PULMONARY ARTERY. HOWARD T. KARSNER, Arch. Int. Med. **51**:367, 1933.

Productive-cicatricial syphilitic disease of the pulmonary artery, i. e., of the Dohle-Heller type, is extremely uncommon, but occurs often enough to justify its consideration in clinical diagnosis. Eleven cases, anatomically proved by reasonably liberal interpretation, are now on record. Eight of these would be regarded as clearly established by the most exacting criteria. Gummatous types of lesion about equal the productive-cicatricial type in incidence, but this ratio may be changed as additional cases are reported. The proportion of productive-cicatricial to gummatous lesions is markedly higher in the aorta than in the pulmonary artery. All cases of productive-cicatricial lesions in the main stem of the pulmonary artery show local or diffuse dilatation of the vessel. Associated disease of the smaller pulmonary arteries is by no means constant, but all cases have shown some degree of cardiac hypertrophy. Thrombosis is more frequently associated with productive-cicatricial lesions of the pulmonary artery than with the same lesions of the aorta. The anatomic character of the lesion, as it affects both the aorta and the pulmonary artery, is essentially the same, although it is probable that in the pulmonary artery the internal elastic lamina is more severely affected than in the aorta. Both the aorta and the pulmonary artery show productive-cicatricial lesions in about half of the cases, but this does not of necessity mean that the disease in one vessel is an extension from the lesion in the other vessel. The disease affects the sexes about equally, and has been found at the age extremes of 28 and 58 years, with the maximum incidence in the sixth decade. It is associated with cardiac difficulties and ultimately congestive heart failure, and may exhibit a fairly characteristic roentgenogram. The most frequent murmur is a systolic murmur over the pulmonic area. Occlusion of a main branch of the pulmonary artery appears to have some relation to development and progress of pulmonary tuberculosis on the affected side.

AUTHOR'S SUMMARY.

HYPERPLASTIC SCLEROSIS OF THE PULMONARY ARTERY AND ARTERIOLES. MARTIN J. SOKOLOFF and HAROLD L. STEWART, Arch. Int. Med. **51**:403, 1933.

A case of hyperplastic sclerosis of the pulmonary artery and arterioles is reported. As an etiologic factor syphilis was as well ruled out as possible. The history was not significant. The patient had no scars on the penis, and he had never had antisyphilitic treatment. The Wassermann and Kahn reactions of the

blood were negative. The patient's wife had had five children and no miscarriages. All the children were living and healthy, and none showed any stigma of hereditary syphilis. There were no suggestive clinical manifestations, and roentgen studies of the patient's skeleton revealed no syphilitic lesions. After death no gross or microscopic evidences of the disease were found in the tissues. No spironemas could be demonstrated, although diligently searched for. In the sections of the pulmonary artery and arterioles there were no newly formed capillaries, round cell infiltrations in the adventitia and media or proliferative endarteritis of the vasa vasorum, characteristic of syphilitic arteritis. The associated factors of etiologic importance were a prior attack of influenzal pneumonia, increased epinephrine in the circulating blood, stimulation, probably reflexly, of the nerves supplying the pulmonary artery and asthma. The term "Ayerza's disease" should not be used synonymously with sclerosis of the pulmonary artery, since the pathologic process may be present for years before the complex of symptoms which Ayerza described make their appearance.

AUTHORS' SUMMARY.

PALATAL MYOCLONUS. WALTER FREEMAN, Arch. Neurol. & Psychiat. **29**:742, 1933.

Freeman describes two cases of rhythmic movements of the pharynx and soft palate which could be interrupted momentarily by phonation and which did not interfere with speech. One patient was alcoholic, with a blood pressure of 244 systolic and 124 diastolic; the other one had marked arteriosclerosis. The pathologic changes were numerous. The most interesting lesions were found in the central tegmental fasciculus and the homolateral olivary body; there was "hypertrophic" degeneration of the homolateral olivary body.

G. B. HASSIN.

ALCOHOLIC ENCEPHALOPATHY. LAURETTA BENDER and PAUL SCHILDER, Arch. Neurol. & Psychiat. **29**:990, 1933.

The twenty-four cases of chronic alcoholism reported by Bender and Schilder represent a variety of syndromes, such as Korsakoff's polyneuritic psychosis, delirium, cerebellar manifestations, acute catatonic states and other forms. Some of the cases have been studied morphologically. The authors concede that the term polioencephalitis, which means an inflammatory condition, is not justified by the type of the histologic changes, and that "alcoholic encephalopathy" is more suitable. The histologic changes described by them were considered constant—ependymitis and subependymal gliosis with a manifest if not exclusive involvement of the gray matter, especially around the ventricles and at the base, including the mamillary bodies. Other striking changes were vascular—hyperemia of the blood vessels, proliferation of the capillaries and occasionally hemorrhages. According to Bender and Schilder, the cause of the central nerve changes is in the spinal fluid, which is filled with toxins.

G. B. HASSIN.

DIFFUSE SCLEROSIS WITH PRESERVED MYELIN ISLANDS. K. LÖWENBERG and T. S. HILL, Arch. Neurol. & Psychiat. **29**:1232, 1933.

The authors' patient was a man, aged 54, who had been sick for eleven years with signs of lesion of the pyramidal tract, intention tremor of the upper extremities, unsteady gait and mental deterioration, which was progressive. There was diffuse sclerosis, as seen in Schilder's disease, with numerous perivascular islands of preserved myelin, as described in Pelizaeus-Merzbacher's disease. There was also demyelinization of the optic thalamus and globus pallidus, while the medulla and pons were normal. The cerebellum was only slightly involved. As the case clinically was not one of Pelizaeus-Merzbacher's disease, the authors classify it as a subdivision of diffuse sclerosis.

G. B. HASSIN.

MORPHOLOGIC CHANGE IN FAT TISSUE PRODUCED BY INSULIN IN MALNUTRITION. H. BLOTNER, J. A. M. A. **100**:1235, 1933.

After a patient suffering from nondiabetic malnutrition had gained 21 pounds (9.5 Kg.) on insulin treatment, the depth of the subcutaneous fat of the abdomen had increased from 3 to 16 mm., and the average diameter of each fat cell from 35 to 79.8 microns. Before the administration of insulin the fat cells appeared small with a thick wall. After the gain in weight, the fat cells became large and distended so that the walls looked like a thin membrane. There appeared to be an increase in the deposit of fat.

FROM AUTHOR'S SUMMARY.

CONGENITAL HEART BLOCK. W. M. YATER, J. A. LYON and P. E. MCNABB, J. A. M. A. **100**:1831, 1933.

A review of the literature reveals records of forty-four acceptable cases of congenital heart block, including the present report. Certain criteria have been applied in collecting these cases. In only five cases have necropsies been performed, and in only three of these have histologic studies of the conduction system been made. A congenital defect of the bundle of His appears to be the cause of this condition in most cases. The most common clinical diagnosis is patent interventricular septum. In the case here reported, there was a defect in the interventricular septum with almost complete congenital absence of the bundle of His. The case is of great significance not only as a completely studied one of congenital heart block, but also as an example of the rarest form of interventricular septal defect.

AUTHORS' SUMMARY.

LESIONS OF THE NERVOUS SYSTEM IN RATS ON A DIET LOW IN VITAMIN A. H. M. ZIMMERMAN, J. Exper. Med. **57**:215, 1933.

Under the conditions of these experiments, which consisted essentially of maintaining rats on a ration adequate in all dietary essentials as far as is known except vitamin A, the following changes were produced in the nervous system: Degeneration of the medullary sheaths of the brachial plexuses and sciatic nerves, and less often of the vagus nerves. Such lesions were not found in the optic nerves. Degeneration of the medullary sheaths of the sensory tracts on the periphery of the spinal cord and in the posterior columns. Much less frequently similar lesions were found in both the crossed and the uncrossed pyramidal tracts. Changes of the same nature were found in the posterior nerve roots and less frequently in the anterior nerve roots of the spinal cord. Evidence was adduced to indicate that the changes in the sensory tracts of the spinal cord followed those in the posterior nerve roots. With the onset of muscular weakness and incoordination in these animals anatomic changes like those just described were found at necropsy, but they were not present for any appreciable period preceding the onset of these clinical signs. For a short but undetermined period following clinical signs of recovery from the nervous disease, marked lesions were still present in the nervous system at necropsy. These lesions in the nervous system were produced by a ration containing no cereals which might have contributed a "toxic" substance to account for the degeneration of the myelin sheaths. Neither does a deficiency in unsaturated fatty acids appear to have played a rôle in their development.

AUTHOR'S SUMMARY.

THE SUBCUTANEOUS NODULES IN RHEUMATIC FEVER AND RHEUMATOID ARTHRITIS. M. H. DAWSON, J. Exper. Med. **57**:845, 1933.

A comparative study on the subcutaneous nodules in rheumatic fever and rheumatoid arthritis was presented as part of an investigation which has been conducted in this clinic on the relationship that exists between these two clinical entities. It is believed that the present study has shown that these subcutaneous nodules are highly characteristic of the two diseases and that they represent different phases of the same fundamental pathologic process. However,

it should be pointed out that the presence of closely related or even identical lesions in two separate clinical entities cannot be considered as valid evidence in support of the hypothesis that the two diseases are etiologically related. Comparative clinical studies on the relationship of rheumatic fever and rheumatoid arthritis will be presented in a succeeding communication. These studies, as well as serologic investigations on the two diseases which have been reported elsewhere, lend further support to the conception that rheumatic fever and rheumatoid arthritis are intimately related and possibly different responses of affected persons to the same etiologic agent.

AUTHOR'S SUMMARY.

OXYCEPHALY IN MAN AND RABBIT. H. S. N. GREENE, J. Exper. Med. **57**:967, 1933.

Craniosynostosis and the pathologic complex with which it is associated in the rabbit are not identical with the abnormalities seen in man; but apparently the two sets of conditions have enough in common to warrant the conclusion that they probably have a common mode of origin. The evidence so far obtained shows that in the rabbit these conditions are hereditary. The factors for the production of the cranial abnormalities are widespread, but the occurrence of deformities is comparatively rare in a general animal population. Inbreeding and selective matings, however, tend to increase their frequency, and also the frequency of other abnormalities with which they may be associated, either by chance or by close genetic relationship in the germ plasm. In this way, a pathologic complex is built up and perpetuated. So far as the cranial abnormalities are concerned, it seems now that oxycephaly and related conditions are variations resulting from a displacement or division of primary ossification centers controlled by hereditary factors.

AUTHOR'S SUMMARY.

THE LESIONS CAUSED BY CERTAIN NEUROTOXIC PHENOL ESTERS. R. D. LILLIE and M. L. SMITH, Nat. Inst. Health Bull., no. 160, 1932, p. 54.

A study has been made of the lesions of the nervous system of cats produced by a group of phenol esters which give rise to characteristic neurologic symptoms after a latent interval of several days' duration.

The compounds producing such delayed neurotoxic action in cats are triphenyl phosphate and triorthocresyl phosphite in addition to the previously described triorthocresyl phosphate.

It is shown that the lesions produced in cats by triphenyl phosphate are mainly a degeneration of the nerve cells in the acute type of poisoning, and degeneration of the peripheral nerves in the subacute types of poisoning.

The lesions produced by triorthocresyl phosphite are shown to consist of combined system degeneration involving ascending spinocerebellar tracts and descending mesencephalic-pontile-cerebellospinal tracts, in addition to the minor degeneration of the lower motor neuron which is characteristic of the action of triorthocresyl phosphate.

AUTHORS' SUMMARY.

BONE MARROW IN THE LUNGS. G. DOLFINI, Pathologica **25**:38, 1933.

Normal marrow was found in the lumen of the pulmonary artery and its branches in a rabbit which died of tuberculosis.

E. VON HAAM.

CHANGES IN THE PANCREAS IN PREGNANCY. K. ROSENLOECHER, Arch. f. Gynäk. **151**:567, 1932.

In pregnancy the islands of Langerhans are increased in number and size. The epithelial cells of the pancreatic parenchyma also are changed, with an increase in the zymogen granules.

POLYPOID GROWTH OF CERVICAL GLANDS IN THE VAGINA DURING PREGNANCY. HANS ZACHERL, Arch. f. Gynäk. **153**:224, 1933.

In a woman ten weeks pregnant, the vagina presented a peculiar polypoid appearance owing to a growth in the wall of the cervical glands, which was thought to have originated in embryonic remains of epithelium from Müller's duct.

JACOB KLEIN.

LESIONS IN THE PULMONARY ARTERY IN RHEUMATISM. HERMANN CHIARI, Beitr. z. path. Anat. u. z. allg. Path. **88**:1, 1932.

Autopsy revealed in the pulmonary artery mucoid degeneration and swelling of the fibrous stroma, leading in some cases to necrosis of the muscular tissue but never to degeneration of the elastic lamellae. Besides these nonspecific changes focal cell proliferations and infiltrations of the media with large cells were noticed. Chiari regards these cell infiltrations, which were mostly grouped around the vasa vasorum, as specific rheumatic lesions. In these areas the elastic fibrils were torn or completely degenerated. These histologic changes explain the relative pulmonary insufficiency from which these patients suffer during life.

C. ALEXANDER HELLWIG.

HISTOLOGIC CHANGES IN THE BLOOD VESSELS IN ALLERGIC CONDITIONS AND THEIR RELATIONSHIP TO PERIARTERITIS NODOSA. WALTER METZ, Beitr. z. path. Anat. u. z. allg. Path. **88**:17, 1932.

In white rats, the histologic picture of periarteritis nodosa was produced by repeated injections of hemolytic streptococci. The same lesions followed injection of beef serum. Periarteritis is therefore regarded as not always due to bacteremia and local immune reactions, but possibly caused also by proteins. The experiments indicate that the primary cause of periarteritis nodosa is a peculiar reaction of the endothelial cells. The histologic changes in the blood vessels of some of the animals resembled rheumatic lesions.

C. ALEXANDER HELLWIG.

MUCOUS GRANULOMAS IN THE SALIVARY GLANDS AND IN THE MUCOSA OF THE STOMACH. H. HAMPERL, Beitr. z. path. Anat. u. z. allg. Path. **88**:193, 1932.

Mucus is often found in the stroma of the salivary glands and in the mucosa of the pyloric portion of the stomach. These deposits of mucus outside of the lumens of the glands are seldom due to inflammatory destruction of the glands. The most common cause is a traumatic rupture of dilated mucous glands. Around these masses, a peculiar granulation tissue forms which encapsulates and finally resorbs the mucus.

C. ALEXANDER HELLWIG.

MORPHOLOGIC CHANGES IN THE HEMATOPOIETIC ORGANS FOLLOWING ACUTE LOSS OF BLOOD. OSCAR LEWIN, Beitr. z. path. Anat. u. z. allg. Path. **88**:349, 1932.

Rabbits were killed after bleeding at periods varying from five minutes to twenty-four days. Two fifths of the total blood volume was removed. In contradistinction to pernicious anemia, acute loss of blood is not followed by erythropoiesis in other organs than the bone marrow. Only in the spleens of experimental animals was a moderate degree of blood formation noticed. There were no morphologic changes in the suprarenals, kidneys, lungs and thymus gland. In the liver, hypertrophy of the Kupffer cells was observed, and in the lymph glands, a marked proliferation of the reticulo-endothelial cells.

C. ALEXANDER HELLWIG.

EARLY POSTMORTEM CHANGES OF THE MEGAKARYOCYTES IN THE MARROW OF THE RABBIT. W. N. MARGOLIN, Beitr. z. path. Anat. u. z. allg. Path. **88**:362, 1932.

The cytology of the bone marrow of twenty-nine rabbits was studied before and after death. As early as one-half hour after death, pyknosis of the nuclei of the

megakaryocytes could be demonstrated. The number of pyknotic cells increased with the interval between death of the animal and examination. After from four to five hours, all megakaryocytes were pyknotic. At this time all the other cells of the bone marrow showed a normal structure. These findings indicate a marked lability of the megakaryocytes.

C. ALEXANDER HELLWIG.

LIPOID SUBSTANCES OF THE BLOOD PLASMA IN BLOOD VESSELS OF DIFFERENT ORGANS. MAXIMILIAN MANDELSTAMM, Beitr. z. path. Anat. u. z. allg. Path. **88**:377, 1932.

Sudanophil substances are frequently found in the blood plasma, especially in the brain. Of seventy-four brains examined, fifty-five showed positive findings. The lipoid substances are also frequently present in the ovaries, thyroid gland, hypophysis and pancreas, while they are less common in the kidneys and are exceptional in other organs. The presence of sudanophil substances in certain organs cannot be explained on the basis of general abnormal conditions of the whole blood, but is most likely due to particularities in the circulation of the blood. The lipoid substances are most frequently found in the blood vessels that contain exclusively, or almost exclusively, blood plasma. They were most abundant in the organs of patients with acute infections, causing disturbance of the peripheral circulation.

C. ALEXANDER HELLWIG.

ASEPTIC INFLAMMATION AFTER BLOCKING LOCALLY THE RETICULO-ENDOTHELIAL SYSTEM. A. N. TSCHISTOWITSCH, Beitr. z. path. Anat. u. z. allg. Path. **88**:426, 1932.

Trypan blue was injected into rabbits subcutaneously, three times within four days. On the fifth day, at the place of injection, a small piece of sponge was implanted under the skin. The foreign body, together with the surrounding tissue, was removed at different intervals and examined histologically. The histiocytes were laden with coarse particles of the intravital stain, and they were found in the region of the foreign body, but had not invaded the sponge. The histiocytes had apparently emigrated from the surrounding connective tissue and from blood vessels. There were no transitions recognizable between fibroblasts and histiocytes. The endothelial cells were without stain. If in such experiments india ink is used instead of trypan blue the stain is not so readily engulfed by the histiocytes. Large amounts of india ink were found free in the interstitial stroma.

C. ALEXANDER HELLWIG.

HISTOLOGY AND ETIOLOGY OF SUBACUTE YELLOW ATROPHY OF THE LIVER IN CHILDREN. R. BENEKE, Beitr. z. path. Anat. u. z. allg. Path. **88**:538, 1932.

The author gives a detailed microscopic description of the internal organs of a child who died of severe jaundice. In the liver, there was almost complete necrosis of the parenchyma. Only a few fatty, degenerated liver cells were preserved. There was proliferation of the bile ducts and of the interlobular stroma. Chemical examination of the internal organs did not reveal any poison, and the history was not in favor of poisoning as the etiologic factor. The author hypothecates that the necrosis of the liver was possibly caused by an intensive spasm of the hepatic artery.

C. ALEXANDER HELLWIG.

LESIONS OF THE PULMONARY ARTERY DUE TO SYPHILITIC MESAORTITIS. JULIUS F. BUCHALY, Beitr. z. path. Anat. u. z. allg. Path. **89**:1, 1932.

In eighteen of the twenty-eight cases of syphilitic mesaortitis, the pulmonary artery was compressed by aneurysm of the ascending branch of the aorta. Adhesions between the aneurysm and the pulmonary artery may produce pressure atrophy of the wall. In ten cases, the syphilitic inflammation had spread from the aorta

to the pulmonary artery, involving also the media. Five cases showed a perforation of the aorta into the pulmonary artery. The syphilitic inflammation may follow the ductus Botalli.

C. ALEXANDER HELLWIG.

DISEASES OF THE LUNGS IN THE NEW-BORN, ASSOCIATED WITH THE ASPIRATION OF AMNIOTIC FLUID. FRANZ SZLÁVIK, Beitr. z. path. Anat. u. z. allg. Path. **89**:40, 1932.

Aspirated amniotic fluid is commonly found in the lungs of the new-born. The anatomic findings following aspiration of the fluid depend on the chemical constituents and the amount of aspirated fluid, the duration of birth and the length of extra-uterine life. They consist in a more or less severe inflammation of the pulmonary tissue, which by itself, however, is never fatal, but heals as a rule within the first week. Aspiration of infected fluid was observed in a stillborn child, the mother having died of streptococcic metritis. Infection of the lungs in the new-born can be due to aspiration of vaginal secretion during birth, to aerogenous infection or to the aspiration of vomitus. It may lead to fatal lobular pneumonia. In one of the author's cases the pneumococcus type IV was the infecting organism. Fetal atelectasis is recognized by the presence of myelin in the alveoli. It forms from degenerated alveolar epithelium.

C. ALEXANDER HELLWIG.

AN UNUSUAL CASE OF CHRONIC FOREIGN BODY PNEUMONIA CAUSED BY GRAIN DUST. HILDE BÖRGER, Beitr. z. path. Anat. u. z. allg. Path. **89**:135, 1932.

A woman, aged 57, died after she had suffered for nine years from bronchitis and chronic pneumonia. She had contracted the disease while watching, for from two to three hours, the operation of a thrashing machine. At autopsy, both lungs showed marked carnification and scar formation, bronchiectasias and pleural adhesions. Many giant cells were found in the pulmonary tissue surrounding foreign bodies, giving the chemical reactions typical of cellulose.

C. ALEXANDER HELLWIG.

OSTEOSCLEROTIC PSEUDOLEUKEMIA. CHARLES WOLF, Beitr. z. path. Anat. u. z. allg. Path. **89**:151, 1932.

A case of osteosclerosis associated with myelogenous pseudoleukemia was studied macroscopically and microscopically at autopsy. The ribs, sternum, vertebrae and femur showed marked sclerosis of the medullary cavity. The sclerosis is only rarely the final stage of leukemia or pseudoleukemia, but is due to premature atrophy of the bone marrow and compensatory extra-osseous hematopoiesis in the spleen and liver. Since in osteosclerosis the formation of blood cells is active only in the liver and spleen, radiation treatment or splenectomy is contraindicated in pseudoleukemia when roentgen examination of the skeletal system reveals an association with osteosclerosis.

C. ALEXANDER HELLWIG.

THE GERMINAL CENTERS OF FLEMING. A. V. ALBERTINI, Beitr. z. path. Anat. u. z. allg. Path. **89**:183, 1932.

The effect of the x-rays, arsenic and lead on the lymph nodes was studied in guinea-pigs and white mice. Following irradiation, diffuse degeneration was noted in the lymphoid tissue of the primary nodules and in the germinal centers, while after arsenic and lead poisoning, only the cells of the germinal centers were destroyed. The lesions in the germinal centers following poisoning are explained by the particular arrangement of the blood vessels in the lymph nodes. The regeneration of lymphocytes after irradiation is not limited to the germinal centers, but is more active in the primary nodules. Therefore, the theory of Flemming, who regards the germinal centers as hematopoietic organs, is not accepted. In the author's opinion, the germinal centers serve as filters in which various poisons brought with the blood stream from other regions of the body are taken up.

C. ALEXANDER HELLWIG.

SPONTANEOUS CEREBRAL HEMORRHAGE. K. WOLFF, Beitr. z. path. Anat. u. z. allg. Path. **89**:249 and 487, 1932.

The histologic changes in brain tissue and blood vessels after traumatic injuries of the brain were studied to form a basis of comparison with the findings by Westphal, Bär and Schwartz, who advocate the modern functional theory of apoplexy. In traumatic destruction of the brain, necrosis of the wall of the blood vessels was observed only in the regions of hemorrhage. Since these angionecroses develop several hours after the injury, they are apparently not the cause of spontaneous hemorrhage, as Schwartz and others hypothecate. Proteolytic enzymes of leukocytes play an important rôle in these vascular changes; probably, also, ferments liberated from destroyed glia cells act on the wall of the vessel. Fat-laden phagocytes appeared not before thirty-six hours after the injury. On the second day, proliferating spindle cells were found in capillaries, and the first collagenous fibers were demonstrated nine days after the accident.

In three of seven brains in cases of fatal apoplexy, ruptured blood vessels were found in the center of the cerebral hemorrhage. The walls of the ruptured blood vessels showed hyaline sclerosis and defects in the elastic layer. In one case in which all the ventricles were filled with blood a ruptured aneurysm was found. In the brains of elderly people, the arteries of the corpus striatum frequently show severe degeneration of the walls, and a rupture may easily occur when the blood pressure is suddenly increased. The author concludes that the classic doctrine of rupture of the blood vessels as a cause of spontaneous cerebral hemorrhage still holds, and that it is better based on facts than the modern theory of functional circulatory disturbances causing apoplexy.

C. ALEXANDER HELLWIG.

SIGNIFICANCE OF LESIONS OF THE HEART MUSCLE IN ANGINA PECTORIS. FRANZ BÜCHNER, Beitr. z. path. Anat. u. z. allg. Path. **89**:644, 1932.

Large longitudinal sections of the hearts in ten cases of angina pectoris were made, the technic of Christeller being used. In all of the cases in which death occurred shortly after a paroxysm, smaller or larger focal necroses were demonstrated in the muscle of the heart. These necroses were preeminently located in the inner layer of the wall of the left ventricle, involving also the papillary muscle and trabeculae. The size of these lesions increased with repeated attacks. Büchner concludes that ischemia and necrosis of the heart are responsible for the attack of pain. Coronary sclerosis and syphilis can produce angina pectoris without spasm of the blood vessels by interfering with dilatation of the coronary arteries during increased action of the heart.

C. ALEXANDER HELLWIG.

THE CORONARY CIRCULATION AND ANGINA PECTORIS IN RELATION TO CORONARY OCCLUSION. W. KOCH and L. C. KONG, Beitr. z. path. Anat. u. z. allg. Path. **90**:21, 1932.

The histopathology of coronary occlusion was studied by means of serial sections of the involved vessels. The results of such a study of seven selected cases are presented. The Spalteholz method of cleared injection preparations was found useful in the investigation of the coronary circulation in disease of the coronary arteries. Twenty-nine hearts of persons who had had anginal attacks form the basis of an attempt to correlate coronary arterial disease with angina pectoris. Coronary stenosis and occlusion may be due to atheroma, fibrous thickening or thrombosis. The three processes are often combined, but the predominance of one or the other process may yield information relative to the duration and character of myocardial changes. Soft atheroma is the most common cause of occlusion, and may occur suddenly as the result of acute degeneration and swelling of an atheromatous nodule. Calcifying senile atherosclerosis has relatively little harmful effect on the muscle of the heart. Although the occurrence of syphilitic disease of the coronary arterial system is admitted, syphilis is of importance chiefly because

it leads to stenosis of the mouth of the coronary artery as a part of syphilitic involvement of the root of the aorta. The effects of occlusion depend on the collateral circulation that is developed in association with coronary disease. Anastomoses are richest between the right coronary artery and the circumflex branch of the left. The descending branch of the left coronary artery has fewest anastomoses, except at its terminal portion, where it unites with the right coronary artery on the posterior surface of the apex of the heart. Infarction occurs most often, therefore, in the territory of distribution of the left descending artery, but is of least functional significance here. Occlusion occurs most often in the proximal portion of the left descending artery and in the distal portions of the circumflex and right descending arteries. In cases with a history of angina pectoris, infarction was a prominent feature, and usually two main vessels were stenosed or occluded. Stenosis of one vessel with an inadequate collateral circulation through another main branch usually leads to acute cardiac insufficiency rather than to anginal attacks. Syphilitic stenosis of the mouth of a coronary artery is a gradual process, which usually permits the development of an adequate collateral circulation; hence infarction is rare. Since the circulation in such cases is through a single coronary artery, sudden death or anginal attacks may occur as the result of myocardial ischemia if excessive demands are made on the coronary circulation.

O. T. SCHULTZ.

HISTOLOGY OF THE GASTRIC MUCOSA AND ACUTE EROSIONS. H. HAMPERL, Beitr. z. path. Anat. u. z. allg. Path. **90**:85, 1932.

This is a systematic study of the human gastric mucosa undertaken for the purpose of evaluating changes that are frequently seen and of determining whether such changes are inflammatory. Stomachs obtained within six hours after death were examined. A strip of mucosa 1 cm. wide, taken along the lesser curvature of the fixed stomach from the cardia to the pylorus, was rolled into a spiral, embedded and sectioned. Two other strips were taken at right angles to this, one across the fundus, the other across the antrum. Great reliance was placed on the oxidase stain for detecting the presence of leukocytes. A modification of Graeff's oxidase method is given for the selective staining of eosinophilic leukocytes. Eosinophilic and neutrophilic leukocytes were found so constantly in the mucosa as to be physiologic or normal. These cells migrate through the mucosa and were seen in the mucus covering the surface of the epithelium. Only when the presence of such cells is associated with changes in the surface and gland epithelium is it proper to speak of inflammation. Inflammation, or true acute gastritis, usually leads to the formation of minute erosions. In these erosions the surface epithelium is injured and then attacked by the gastric juice. Beneath this superficial zone of necrosis is a second zone of fibrinoid necrosis, and beneath this a zone of leukocytic infiltration in which leukocytes are more numerous than in the normal gastric mucosa. The accumulation of leukocytes is held to be secondary to the necrosis of the more superficial tissue.

O. T. SCHULTZ.

Microbiology and Parasitology

DETERMINATION OF TUBERCLE BACILLEMIA BY LÖWENSTEIN'S METHOD. JOSEPH SCHRAMEK, Beitr. z. Klin. d. Tuberk. **82**:606, 1933.

Apparently tubercle bacillemia is an infrequent occurrence in tuberculosis, for in only 2 of 322 cases of pulmonary tuberculosis in which 522 blood cultures were performed was it possible to demonstrate tubercle bacilli. In none with extrapulmonary complications were they found. Nevertheless, experiments proved that Löwenstein's method is trustworthy in revealing the presence of tubercle bacilli in blood.

AARON EDWIN MARGULIS.

EXPERIMENTAL REINFECTION OF GUINEA-PIGS WITH THE VIRUS OF SPOTTED FEVER. KENSABURO TAKAHASHI and SOYO HOSHIZAKI, Zentralbl. f. Bakt. (Abt. 1) **121**:39, 1931.

Forty-six guinea-pigs were reinfected with the virus of spotted fever at intervals of from one to one hundred and twenty-seven days after they had become free from the fever produced by the first infection. Ten showed symptoms (fever and loss of weight), but the period of incubation was prolonged as compared with the first infection, while the febrile period was irregular and shortened.

PAUL R. CANNON.

EXPERIMENTAL STUDIES OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM. T. TANI, K. SAITO and H. FUNADA, Zentralbl. f. Bakt. (Abt. 1) **123**:219, 1932.

Rabbits (thirty) were infected intratesticularly, subscrotally or suboccipitally with syphilitic material (passage strain "VIII") and were treated with neoarsphenamine intravenously from thirty-five to five hundred and thirty-nine days after infection. Keratitis developed in five animals, whereas the others remained symptomless. A negative Wassermann reaction in the serums of eight animals became positive but that of the spinal fluids remained negative. Spirochetes were regularly demonstrable in the blood until after the third injection of neoarsphenamine, but could not be found in the spinal fluid at any time. Spirochetes were demonstrated in the blood, spleen, testes and lymph nodes of all but one of seven rabbits thus examined which had been infected for an average period of six hundred and sixty-five days and had had from eight to nine injections of neoarsphenamine. Spirochetes were found in the brain in only one of the six rabbits whose brains were examined.

Fourteen rabbits infected intratesticularly or suboccipitally were treated with neoarsphenamine and were then inoculated suboccipitally on several occasions with homologous or heterologous strains of syphilitic virus. Four normal untreated rabbits were inoculated similarly. Keratitis or orchitis developed in the latter, whereas the previously infected and treated animals showed no symptoms. The negative Wassermann reaction of the blood serum became positive in three of the four normal rabbits and in three of the fourteen previously infected and treated ones. Spirochetes were demonstrable in the blood of the control animals after the second injection, but could not be found in the treated animals until after the fourth injection. They were not demonstrable in the spinal fluid in either group. Neither could spirochetes be found in the brains of the rabbits from either group.

The authors conclude that the rabbits previously infected and treated with neoarsphenamine showed a definite acquired immunity, although this could be overcome by repeated superinfections. The authors believe that the immunity of the central nervous system to syphilis is unusually high in the rabbit, but they cannot explain this resistance.

PAUL R. CANNON.

SOURCE OF THE GUARNIERI BODIES. E. PASCHEN, Zentralbl. f. Bakt. (Abt. 1) **124**:89, 1932.

The author gives opinions as to the source and nature of the Guarnieri bodies in the skin and eye. He believes that many of the cell inclusions are simply pyknotic nuclear fragments, nucleoli and chromatin masses from degenerating leukocytes, but that the bodies which actually contain the virus are very small elements immediately around the nucleus in cells at the site of inoculation.

PAUL R. CANNON.

MORPHOLOGIC DEMONSTRATION OF THE SMALLPOX VIRUS IN TISSUE CULTURE. E. G. NAUCK and E. PASCHEN, Zentralbl. f. Bakt. (Abt. 1) **124**:91, 1932.

The authors injected vaccine virus into rabbits' testicles and subjected the latter to tissue culture. They observed a direct correlation between the numbers of Paschen bodies and the virulence of the cultures. These bodies persisted through thirty-five passages in tissue culture, and in some instances yielded positive results

in the skin of rabbits in a dilution of 1 : 100,000 of the original culture. The authors conclude that these so-called elementary bodies described by Paschen are the carriers of the virus.

PAUL R. CANNON.

EFFECTS OF INHALATION OF EUCALYPTOL ON TUBERCULOUS GUINEA-PIGS INFECTED INTRATRACHEALLY. T. H. AMAKO, Zentralbl. f. Bakt. (Abt. 1) **124**: 95, 1932.

Guinea-pigs were infected intratracheally with tubercle bacilli and were subjected in enclosed cages to treatment by inhalation of eucalyptol until the death of the animals. This treatment had no appreciable effect on the length of life of the infected animals as compared with control animals similarly treated.

PAUL R. CANNON.

CULTIVATION OF THE VIRUS OF SPOTTED FEVER. ILCHUN YU, Zentralbl. f. Bakt. (Abt. 1) **124**:181, 1932.

Passage virus derived originally from the blood of a patient with spotted fever was injected intraperitoneally into a guinea-pig, and the testes and tunica vaginalis were removed aseptically from twenty-four to forty-eight hours later, cut into small pieces and grown in tissue culture. The virus persisted for seventy days, through six generations, in vitro and was demonstrable both in the tissue and in the culture fluid itself. Rickettsial bodies were never found in the tissue culture except when embryonic extract, from embryos of guinea-pigs convalescing from a previous infection, was used.

PAUL R. CANNON.

Immunology

GROUP-SPECIFIC QUALITIES IN SALIVA AND OTHER BODY FLUIDS AND DEMONSTRATION OF TWO TYPES OF ELIMINATORS OF THE GROUP-SPECIFIC QUALITIES. HAKARU SASAKI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:101, 1932.

All persons can be divided into two groups: (*a*) those who eliminate the group qualities A, B or O in the saliva, gastric juice, milk or urine and (*b*) those who do not eliminate them. The ability to eliminate is constitutional and does not depend on the blood group. In persons of group A B the two factors always behave identically. The presence of the group qualities in the secreta was established by means of the inhibition of agglutination and hemolysis. The inhibition of the specific anti-O agglutination (in absorbed ox serums) is brought about by secretions of all four groups. The qualities M and N were not demonstrated in the saliva. There was no demonstrable relation between the presence in the saliva of the ferment which is able to destroy blood group qualities and the presence or absence of elimination of the group qualities.

I. DAVIDSOHN.

INHERITANCE OF SEROLOGIC TYPES OF ELIMINATORS OF GROUP-SPECIFIC SUBSTANCES. F. SCHIFF and H. SASAKI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:129, 1932.

The authors made a study of 68 families with 215 children and 144 twins. The eliminators of the qualities A, B and O in saliva were designated as S; those failing to eliminate as s. The S and s qualities are inherited as a mendelian pair and are fully developed in infants. The s quality was encountered more frequently in the O group than in the others.

I. DAVIDSOHN.

PURIFICATION OF GROUP SUBSTANCE A OF HUMAN RED BLOOD CELLS. F. OTTENSOOSER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:140, 1932.

The group-specific substances are contained in the stroma and not in the hemoglobin. The inhibiting effect on an antisheep hemolysin produced by injection of

human red blood cells of group A, according to the procedure of Brahn and Schiff, was found the most sensitive reaction for the study of the substance A. The stroma containing the substance A contains no less of the group-specific substance than the red blood cells from which it was prepared. A method of preparation of stroma of high purity with only from 0.5 to 2.5 per cent of hemoglobin is described. Extracts prepared from stroma with a highly diluted sodium hydroxide solution contained a more active A substance than the original stroma, which indicates that the alkali had an activating effect.

I. DAVIDSOHN.

PRODUCTION, PROPERTIES AND ACTION OF CONCENTRATED TOXOID. C. SIEBENMANN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:167, 1932.

Acetic acid and barium sulphate were used for the precipitation. Only one of three horses gave a satisfactory antitoxin. All three horses showed various degrees of renal damage, and one of them eliminated diphtheria antitoxin in the urine. Rises in temperature were frequent. Siebenmann recommends the exclusion of horses with fever at the beginning of the treatment. There is no reason to recommend replacement of strong unconcentrated toxoid by the concentrated toxoid, but weak toxin can be made useful by concentration.

I. DAVIDSOHN.

INDUCTION OF CHANGES IN THE SPECIFICITY OF IMMUNE SERUMS BY CHEMICAL TREATMENT. F. BREINL and F. HAUROWITZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:176, 1932.

Horse immune serums against paratyphoid bacilli A and B and against dysentery Y and Flexner bacilli were treated according to Landsteiner's method with atoxyl, aniline, metanilic acid, iodine, formaldehyde and sodium hydroxide. The serums were tested before and after treatment for the specific and group agglutinins, and also with an antihorse precipitating serum for species specificity. The antibodies were influenced in different degrees. Iodine destroyed agglutinins but was less harmful to precipitinogens; formaldehyde made the serums highly specific and left the precipitinogens more or less intact. The other procedures had a uniformly destructive effect on the antibodies as well as on the species specificity. Diphtheria antitoxin and typhus convalescent serum were uniformly damaged by all procedures. The conception that antibodies are proteins or that they are closely associated with proteins is supported by a chemical analysis of the experimental results. The aromatic groups of the protein molecule are no less important for the specificity of the antibody than they are for the antigen.

I. DAVIDSOHN.

INACTIVATION OF COMPLEMENT WITH THE POISON OF THE SNAKE BOTHROPS. OTTO BIER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:187, 1932.

The poisons of the snakes Bothrops jararaca (Wied) and Bothrops atrox (L) destroyed the thermostable fourth component of complement. The time of inactivation had to be strictly adhered to. The action on less diluted and on heated serum is different from the action of diluted ammonia, which is known to destroy the fourth component of complement.

I. DAVIDSOHN.

THE FLOCCULATION TIME DURING THE IMMUNIZATION OF HORSES FOR DIPHTHERIA ANTITOXIN. A. J. VAN DEN HOVEN VAN GENDEREN and C. A. KRAMERS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:280, 1932.

Five horses were treated with diphtheria anatoxin. The flocculation time became stationary for many months after an initial rise. After a longer interval between injections, their renewal was in some cases followed by a shortening of the flocculation time; however, there was no quantitative relation between the new flocculation time and the rise of the antitoxin titer. Both the in vivo and the in

vitro titers were determined; the former not infrequently had a tendency to drop below the latter in the course of the treatment. The in vivo titer had a tendency to sink following a large venesection.

I. DAVIDSOHN.

NATURAL HEMAGGLUTININS IN SNAKES AND OTHER COLD-BLOODED ANIMALS. A. DO AMARAL and D. V. KLOBUSITZKY, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:315, 1932.

Eight species of snakes, two species of turtles and one species of lizards, as well as the horse, the sheep and the goat, were studied. No auto-agglutinins or iso-agglutinins were found in the plasma of the cold-blooded animals. The plasma of some of the snakes and of one turtle agglutinated occasionally the erythrocytes of one of the lizards. The plasma and erythrocytes of the snakes and the horses did not act on each other. The erythrocytes of one of the species of turtles (Bufo marinus) were agglutinated by the plasma of the horses, and, in turn, their plasma agglutinated the erythrocytes of some horses.

I. DAVIDSOHN.

THE SPECIFICITY OF FIBRIN. HANS J. FUCHS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:343, 1932.

The precipitate obtained from serum with 2.5 per cent trichloracetic acid, followed by washing, possessed the same qualities as the purified fibrin which Fuchs employed previously as the reagent in his test for cancer. The test is based on the observations of Freund and Kaminer that normal serum dissolves carcinomatous cells and on those of Weinberg that the normal serum shows an increase of nonprotein nitrogen after such treatment. Fuchs demonstrated the presence of the tumor-specific substance in the fibrin and in the precipitate of the serum. On the basis of 3,400 cases he found it to be tumor specific but not to permit the differentiation of various types of tumors and of their origin. The serum of patients with cancer had no such effect on the tumor substance. Occasionally a decrease instead of an increase of the nonprotein nitrogen was noted in the mixture of serum of patients with cancer and fibrin or precipitate of such patients. This is, according to Fuchs, a sign of developing immunity. The fibrin and the precipitate contain immune bodies of the serum as well as antigenic substances. Antisheep hemolysin was similarly demonstrated in the serum precipitate of immunized rabbits.

I. DAVIDSOHN.

ORIGIN OF DIPHTHERIA TOXIN. RICHARD PRIGGE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:421, 1932.

Dried, washed and pulverized diphtheria bacilli have the same toxic effect on guinea-pigs as toxin obtained from cultures. Their toxicity is destroyed by heating at 100 C. and by neutralization with antitoxin. While it was impossible to liberate toxin from the bacilli by means of high pressure, large quantities of the toxin were liberated by suspension in physiologic solution of sodium chloride. The yield of toxin decreased rapidly with repeated washings, but if the bacterial bodies were left alone for a few days considerable quantities of toxin could again be obtained by washing with physiologic solution of sodium chloride. After repeated washings at proper intervals, the bacterial bodies were found to contain only traces of toxin. The ease with which the toxin passes from the bacterial body into the surrounding medium suggested to Prigge that it is a part of the bacterial body and that after the death of the cell it is liberated gradually with progressive disintegration. Prigge checked and disproved the hypothesis of Dernby and Walbum that the diphtheria toxin is not produced in the bacteria but is a result of the action of enzymes, liberated from dead bacterial bodies, on albumoses and peptones of the medium.

I. DAVIDSOHN.

STIMULATING EFFECT OF TAPIOCA ON THE PRODUCTION OF DIPHTHERIA ANTITOXIN. E. W. SKROTZKY, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:443, 1932.

The work was undertaken to check the contradictory reports concerning the effect of tapioca on immunologic response, first published by Ramon. The ability of tapioca to adsorb diphtheria toxin could not be observed in the animal experiment. Precipitation between toxin which was mixed with tapioca and antitoxin was distinctly more marked than that which occurred when the untreated toxin was used. The increase was assumed to be nonspecific. The local irritating effect of tapioca on the skin was rather mild; necrosis was not observed. The formation of a depot in the inoculated area and the increase of the antigenicity recommend the employment of tapioca as an admixture to diphtheria toxin.

I. DAVIDSOHN.

GROUP-SPECIFIC DIFFERENTIATION OF PLACENTAL ORGANS. HORST REICH, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:449, 1932.

The decidua parietalis (vera) of mothers belonging to group A always contains the quality A, while the decidua basalis contains little of it. The amnion of children belonging to group A contains the quality A, but in varying amounts. The chorion frondosum in the cases of children of group A lacks the quality A. The quality A can be removed from the tissues by proper procedures. In a few cases in which the mother belonged to group O or B and the child to group A the quality A was found in the decidua parietalis. Reich explains that finding by imbibition from the amnion. The placenta proper is considered a zone of group neutrality. The discovery by Schiff and Weiler of a ferment in the placenta which is able to destroy the blood group-specific qualities may be the explanation.

I. DAVIDSOHN.

ACTION OF FECAL EXTRACTS ON ANTIGENS. M. EISLER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:472, 1932.

Extracts of human feces destroyed blood group-specific substances in human red blood cells and secretions and in Witte's peptone. Such extracts did not affect precipitinogens of human and horse serum and of paratyphoid bacilli and the Forssman antigen of horse kidney. Fecal extracts of the sheep, dog and rat destroyed the substance A of Witte peptone, but the destructive effect of the feces of rabbits and goats was slight or absent.

I. DAVIDSOHN.

SOME QUALITIES OF THE THERMOSTABLE COMPONENTS OF COMPLEMENT. T. MISAWA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:477, 1932.

The report of Gordon, Whitehead and Wormall concerning the presence of the third component in the globulin, and of the fourth in the albumin, fraction was confirmed in principle, although such clearcut separation was seen only occasionally. In most cases each component was predominantly associated with the corresponding fraction. The separation of the thermolabile midportion and end-portion is entirely independent of the separation of the thermostable fractions. The destruction of the third component by cobra poison does not take place if the complement was previously heated at 55 C., or if it was treated with hydrochloric acid in a hypertonic solution. The destruction of the fourth component by ammonia is not affected by these procedures.

I. DAVIDSOHN.

ANAPHYLAXIS AND IMMUNITY. S. METALNIKOV, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:488, 1932.

The defense reactions of immunity and the shock of anaphylactic hypersensitiveness are essentially identical phenomena of the hypersensitive organism.

The hypersensitiveness is brought about by the first introduction of the antigen, and the different manifestations of the two conditions are explained by the small quantity of the reintroduced antigen in the case of immunity reactions and by the excessive dose in the case of anaphylaxis. Caterpillars of Galleria melonella were inoculated with 0.1 cc. of a culture of Vibrio cholerae. After from twenty-four to forty-eight hours a reinjection of a minimum lethal dose had no bad effects, though control insects were killed within from fifteen to twenty-four hours. Caterpillars similarly immunized were reinoculated with large doses of Vibrio cholerae. They became very ill within from ten to fifteen minutes and died from two to three hours later. Normal caterpillars given similar large doses appeared normal in from two to three hours and died from eight to ten hours after the injection. An experiment yielding identical results was made with guinea-pigs treated with Vibrio cholerae.

I. DAVIDSOHN.

THERMOSTABILITY AND THE NATURE OF ANTIBODIES. L. A. SILBER and M. W. DEMIDOWA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:504, 1932.

Increase in the thermostability of antibodies is due to prevention of coagulation and of denaturing of proteins. Prevention of coagulation preserves only the H agglutinins; prevention of the denaturing action of heat preserves the H and the O agglutinins. Dilution of the serum has an anticoagulating effect, while saccharose acts as an antidenaturant. Heat exerts at the same time a denaturing effect on proteins and a destructive effect on antibodies. The two effects are similar and parallel. This suggests that antibodies are protein substances or are closely associated with them.

I. DAVIDSOHN.

FURTHER STUDIES ON THE THERMOSTABILITY AND NATURE OF THE COMPLEMENT. L. A. SILBER and A. S. SCHAFRAN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:514, 1932.

Antidenaturants, such as saccharose, dextrose, maltose and sorbite, in certain definite concentrations, increased the thermoresistance of complement, enabling it to stand heating at 56 C. for fifteen minutes. Anticoagulants without, or with slight, antidenaturing properties failed to protect. A close relationship was observed between the destruction of the complementary properties of the serum and the denaturing of its proteins.

I. DAVIDSOHN.

SYNTHETIC LECITHIN AND ITS ANTISERUMS. HERTA MAIER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:1, 1933.

The serums of rabbits treated with a mixture of distearyl lecithin and hog serum possessed specific antibody functions with the homologous antigen and with a commercial lecithin. A lecithin antiserum also reacted with distearyl lecithin. The distearyl lecithin antiserum precipitated with the commercial lecithin but not with the homologous antigen. The serum reacted strongly and selectively with lecithin obtained from the brain of the ox, sometimes even more strongly than with the homologous antigen, thus making it advisable to employ the brain preparation, particularly after the addition of cholesterol, as a reagent for the testing of distearyl lecithin antiserums. This reactivity was not organ specific, as shown by tests with alcoholic extracts of brain tissue. Sometimes, though not regularly, complement was fixed with the acetone-insoluble fraction of the alcoholic brain extract.

I. DAVIDSOHN.

INFLUENCE OF FORMALDEHYDE ON DIPHTHERIA TOXIN. I. S. SCHMIDT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:27 and 323, 1933.

The rapidity with which diphtheria toxin changes into anatoxin increases with the concentration of formaldehyde, and with a rise in temperature and in the

hydrogen ion concentration, and the change may become almost immediate with high concentrations of formaldehyde. Together with the change in toxicity, which is not reversible, the resistance to heat, acids, alkalis and other chemical influences increases. Because of the marked thermal instability of purified toxin, Schmidt suggests first keeping the mixture of purified toxin and formaldehyde at 0 C. and then increasing the temperature, or changing the raw toxin into anatoxin and then purifying the finished product. The immunizing properties of anatoxin depend not only on its flocculating ability but on other, as yet undetermined, factors which may be due to the presence of extraneous inhibiting or stimulating substances in the medium. The technic employed in the treatment of toxin with formaldehyde may also be responsible.

I. DAVIDSOHN.

ACTION OF FORMALDEHYDE ON DIFFERENT ANTIBODY FUNCTIONS. H. BRAUN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:46, 1933.

The various antibody functions of different types of immune serums are influenced differently by formaldehyde. In a Forssman heterophilic antiserum the complement-fixing antibodies were destroyed by certain concentrations of formaldehyde which did not affect the precipitins. The effect on an antisheep serum was similar; in this case the agglutinins proved more resistant than the hemolysins. The effect of formaldehyde was directly proportionate to the duration of its action, to its concentration and to the temperature. In syphilitic serums the complement-fixing and the precipitating qualities were affected in a uniform manner. In protein antiserums (produced by injection of serum) the complement-fixing qualities were more resistant than the precipitins. The species-specific heteroagglutinins anti-O in rabbit immune serums were less resistant than the group-specific agglutinins directed against the quality A.

I. DAVIDSOHN.

THE FRACTIONS OF COMPLEMENT: I. THE FOURTH AND FIFTH FRACTIONS. T. TODA and B. MITSUSE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:62, 1933.

Toda and Mitsuse confirm the existence of the fourth fraction of complement. This fraction is destroyed by chloroform and ether as well as by ammonia and its salts. The technic of preparation of different fractions is described. A fifth fraction was demonstrated, which is destroyed by benzine, and which possesses the highest degree of thermostability of all the fractions, as it stands boiling for one hour. It is recovered in the albumin fraction of the serum, as is the fourth fraction, while the third fraction is attached to the globulin. A comparison of the thermal resistance of the different fractions showed that it rose from the mid-fraction through the end-fraction—from the third to the fourth and to the most resistant fifth fraction. The poison of a viper destroyed the fourth fraction. The same effect was produced by cadmium chloride, known to react readily with lecithin. The fractions destroyed by ether and benzine could be recovered by evaporating the extract and adding the residue to the inactivated serum. The fourth and fifth fractions are assumed to be lipoidal or closely attached to lipoids.

I. DAVIDSOHN.

INFLUENCE OF PHYSICOCHEMICAL FACTORS ON HETEROPHILIC ANTIBODIES. ILSE SACHS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:122, 1933.

Rapid dilution of the antigenic extract renders it more sensitive if small amounts of the extract are employed, and particularly if the time of the primary incubation is shortened. When larger quantities of the extract are used, the fractional dilution may, under proper conditions, offer advantages. The heterophilic precipitation reaction was considerably increased by incubator temperature. Shaking of the test tubes hastened and intensified the precipitation, but did not affect the

complement fixation. The complement-fixing and hemolytic functions of heterophilic and immune serums, which under the usual conditions are lost by heating to 65 C., could be demonstrated by the use of a very slow fractional dilution of the extract, by prolonged (eighteen hour) icebox fixation of the mixture (antigen, heated antiserum and complement) and by lowering of the sodium chloride concentration (0.6 per cent) during the primary incubation. The precipitating antibodies were absorbed from the fresh and from the heated serum by sheep cells but not by ox cells. After treatment with heated heterophilic antiserum, heterophilic antigen lost the ability to react with proper unheated serums, although the heated serums lack the ability to fix complement with a heterophilic antigen. The observation that normal rabbit serum has an inhibitory effect on the heterophilic antigenic extract similar to that of the heated heterophilic antiserum does not permit the attribution of a specific character to the phenomenon. Sachs concludes that the observation does not affect the unitary conception of the antibodies, but that it does not lend support to the other hypotheses.

I. DAVIDSOHN.

B ANTIGEN AND B ANTIBODIES IN MAN AND ANIMALS. V. FRIEDENREICH and S. WITH, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:152, 1933.

Human anti-B agglutinins are absorbed by human red cells of type B and by rabbit red cells. The anti-B agglutinins of rabbit immune serum are not absorbed by rabbit red cells. The two B qualities and the two anti-B agglutinins are not identical. The human red cells of type B contain a B_2 quality, which they have in common with the rabbit red cells, and a B_1 quality, which is not shared. Thus the rabbit immune serum produced by injection of human B erythrocytes contains only anti-B_1 agglutinins. The titers of the anti-B_1 agglutinins in human serums vary considerably. The B quality was found in the red cells of the dog, rat, pig, and guinea-pig, but not in those of the ox, sheep, goat, chicken and pigeon. The B_1 quality was not found. Anti-B_2 agglutinins were found in the blood serum of chickens, while in all other species only anti-B_1 agglutinins were present.

I. DAVIDSOHN.

COMPARATIVE COMPLEMENT-FIXATION TESTS IN LEPROSY, TUBERCULOSIS AND SYPHILIS. G. KORNEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:207, 1933.

The technic for the preparation of a leproma antigen is described. It was somewhat more specific than various syphilitic antigens and the antigen of Witebsky-Klingenstein-Kuhn, for which a marked specificity in the complement-fixation test for tuberculosis was claimed by its authors. The antigens employed for the serologic diagnosis of syphilis and of tuberculosis did not permit a differentiation of leprosy in the presence of simultaneous tuberculous or syphilitic infections.

I. DAVIDSOHN.

ANTIGENIC QUALITIES OF BACTERIA TREATED WITH ULTRAVIOLET RAYS. ST. GÄRTNER and J. SZATHMÁRY, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:256, 1933.

Typhoid and paratyphoid B bacilli and Vibrio aquatilis were exposed for sixty minutes and one hundred and twenty minutes to ultraviolet radiation. Their ability to produce agglutinins in rabbits decreased progressively with the exposure; the loss of antigenic qualities was proportionate to the sensitiveness of the bacterial species to irradiation, which was measured by estimation of changes in the turbidity of the bacterial suspension. The specificity and the resistance to thermal and chemical influences were not influenced by irradiation.

I. DAVIDSOHN.

CAUSE OF THE POSITIVE WASSERMANN REACTION IN MALARIA. O. FISCHER and O. D. GÜNSBERGER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:295, 1933.

The frequency of a positive Wassermann reaction during, and for various lengths of time after, malarial attacks is due, according to Fischer and Günsberger, to the development of antibodies against the lipoids of the destroyed red blood cells. An alcoholic extract of erythrocytes reacted in twenty-one of twenty-eight cases with serums of patients with malaria, occasionally but only in low dilutions with syphilitic serums and not at all with normal Wassermann-negative serums. The differentiation between the serums of patients with malaria and with syphilis was possible by the evaluation of the titers obtained in complement-fixation tests with various extracts. A highly sensitive beef heart extract was particularly helpful.

I. DAVIDSOHN.

Tumors

DIFFUSE CYSTADENOMA OF THE PANCREAS. G. C. PARENTI, Pathologica **25**:1, 1933.

A case of diffuse cystadenoma of the pancreas is reported. After a detailed description of the microscopic appearance of the tumor, the conclusion is reached that it originated from aberrant embryonic cell rests.

JACOB KLEIN.

CENTRAL CHONDROSARCOMA OF METAPHYSES. N. GULECKE, Arch. f. klin. Chir. **174**:401, 1933.

The first of the three cases of central chondrosarcoma of a tubular bone near the joint was noticed after fracture of the neck of the femur in a 48 year old woman. Symptoms existed seven years prior to the diagnosis, which was made by biopsy. When shortly afterward the patient died of hemorrhagic pancreatitis no metastases were found in the internal organs. The second tumor, in a 30 year old woman, was treated for some time as tuberculosis of the hip joint. Eight years after the first symptoms the involved portion of the femur was resected. Four and one-half years after the operation the patient died with a local recurrence of the tumor. The third tumor occurred in the humerus of a 58 year old bartender. Fourteen years after the earliest symptoms the upper half of the humerus was resected. Eleven years after the operation the patient was still alive, but had pulmonary metastases and a local recurrence.

The course of a tumor of this type is long and relatively benign as compared with that of a central sarcoma. Because metastases are late, operation should be undertaken even in late stages. The roentgenogram is usually so characteristic as to enable one to make the right diagnosis. In doubtful cases, biopsy is indicated.

C. ALEXANDER HELLWIG.

TWO CASES OF METASTASIZING NEURINOMAS OF THE GASTRO-INTESTINAL TRACT. KURT DENECKE, Beitr. z. path. Anat. u. z. allg. Path. **89**:242, 1932.

The author gives a macroscopic and microscopic description of a pedunculated solitary neurinoma of the duodenum and of a cystic neurinoma of the stomach. In both cases large metastases to the liver were present. No glia fibrils and no axis-cylinders were found in the primary tumors and the metastases. From the histologic structure, the author concludes that these neurinomas originated from the cells of the sheath of Schwann.

C. ALEXANDER HELLWIG.

A CASE OF SO-CALLED CHONDROMA OF THE LUNG. RICHARD PETERS, Beitr. z. path. Anat. u. z. allg. Path. **89**:484, 1932.

In a 69 year old man a large chondroma was found at autopsy, located in the center of the lower lobe of the left lung. It was composed mostly of

cartilaginous tissue, but contained also mucous glands, unstriated muscle, fat and fibrous tissue. The complex tumor was derived from a misplaced anlage of a bronchus.

C. ALEXANDER HELLWIG.

PRODUCTION OF TUMOR-LIKE GROWTHS BY INORGANIC SALTS. ERNST LEUPOLD, Beitr. z. path. Anat. u. z. allg. Path. **89**:542, 1932.

Small papillomas were produced on the tongues of rabbits by injecting into the tissue hypotonic salt solutions. Simple washing of the oral cavity was also effective. The most potent substances were calcium chloride and sodium chloride. When the same substances were injected near artificial wounds in the ears of rabbits, fibroma-like growths appeared in the healing wounds. These tumor-like growths were not autonomous but disappeared spontaneously after cessation of the irritation. Disturbance in the salt concentration of the tissue is regarded by Leupold as an important factor in the etiology of neoplasms.

C. ALEXANDER HELLWIG.

A CASE OF MYOBLASTOMA ASSOCIATED WITH SQUAMOUS CELL CARCINOMA OF THE TONGUE. R. SCHIRMER, Beitr. z. path. Anat. u. z. allg. Path. **89**:613, 1932.

From the tongue of a 36 year old man a tumor was removed which was composed chiefly of very unripe myoblasts without striation. The epithelium covering the tumor had formed a ripe squamous cell carcinoma penetrating into the unripe rhabdomyoma. This myoblastoma is explained on the basis of Cohnheim's theory of misplaced tissue cells. In spite of their embryonal structure, the unripe rhabdomyomas are clinically benign, but they are frequently associated with atypical or cancerous proliferation of the covering epithelium.

C. ALEXANDER HELLWIG.

SUBLEUKEMIC RETICULO-ENDOTHELIOSIS TERMINATING IN RETICULO-ENDOTHELIAL SARCOMA OF THE HUMERUS. H. UNGAR, Beitr. z. path. Anat. u. z. allg. Path. **91**:59, 1933.

A woman, aged 48, whose illness had been characterized by pain in the sacroiliac region and progressive loss of weight and strength, died of lobar pneumonia seven months after the apparent onset of her illness. The first examination of the blood, two months after the onset, revealed a normal leukocyte count and distribution and anemia that led to a tentative diagnosis of pernicious anemia. In subsequent examinations the anemia, which progressed in spite of liver therapy, assumed the characters of a secondary anemia. The leukocyte count remained normal or was only slightly increased, but from 50 to 66 per cent of the cells were lymphocytic, leading to a diagnosis of aleukemic lymphadenosis. The spleen was palpable, but the superficial lymph nodes were not enlarged. At necropsy there was discovered a tumor of the humerus, apparently arising from the periosteum, with multiple metastases to the vertebrae, long bones and ribs, The reticulo-endothelium of the liver, spleen and bone marrow and of some of the lymph nodes was hyperplastic. In the liver and spleen transitions from reticulo-endothelial cells to lymphocytic cells like those of the peripheral blood could be seen. The condition is interpreted as a primary hyperplasia of the reticulo-endothelial system which terminated as a reticulo-endothelial sarcoma of bone. The reticulo-endothelial hyperplasia of the bone marrow is considered the intermediate stage between the generalized reticulo-endothelial hyperplasia and the terminal bone tumor. The case is made the basis of a discussion of the orgin of the blood cells, the author adhering to the trialistic doctrine, although admitting that the active mesenchyme may give rise to the various types of blood cells under abnormal conditions. The relations of this case to other reported examples of reticulo-endothelial hyperplasia are discussed.

O. T. SCHULTZ.

HISTOLOGY OF MIXED TUMORS OF THE SALIVARY GLANDS. W. E. ZYMBAL, Beitr. z. path. Anat. u. z. allg. Path. **91**:113, 1933.

Zymbal's presentation of the histology and cytology of the so-called mixed tumors of the salivary glands is based on the study of fifty-five such tumors of the parotid gland, two of the submaxillary gland and one of the upper lip. Transitions from normal glandular tissue to tumor tissue and from one type of tumor tissue to another were observed. The wide variations in the cellular composition of these tumors, extending from tubular glandular structures of practically normal morphology to solid, carcinoma-like areas, are described and illustrated in detail. The wide variation in the structure of the mitochondrial apparatus of the cells is apparently dependent on differences in the secretory activity of the cells. The metachromatic ground substance of the tumors is the result chiefly of the secretory activity of the cells. The cartilage-like ground substance is derived from degenerated tumor cells, which contain variable amounts of mucus or mucoid material that enters into the formation of the cartilage-like material. In eight of thirteen tumors in which explantation of the fresh tissue was done, the preparations remained viable and exhibited evidences of growth for as long as three weeks, but no proliferating transplantable tissue cultures were obtained. The cellular character of the growing explant is dependent on the cellular character of the tumor tissue that is explanted. The author concludes that the tumors under discussion are purely epithelial in origin and should not be termed mixed tumors.

O. T. SCHULTZ.

EXPERIMENTALLY INDUCED EPITHELIAL PROLIFERATION OF THE RABBIT'S TONGUE. W. RONDORF, Beitr. z. path. Anat. u. z. allg. Path. **91**:176, 1933.

This is a contribution to the conceptions of Leupold, under whose direction the work was done, of the effect of local cellular metabolism, as influenced by certain organic and inorganic substances, on cellular proliferation. The substances used in these experiments were calcium chloride and potassium chloride in solutions of 1:1,000,000 strength, and leucine, glycogen and cholesterol, mostly in solutions of 1:1,000,000 and 1:10,000,000. The quantity injected just beneath the epithelium of the margin of the rabbit's tongue was 0.05 cc. This quantity and the strengths of the solutions are reminiscent of the homeopathic procedure of "proving" of drugs. The injections were repeated at intervals of from two to eight or ten days. Judged by the size and number of papillomas that developed, calcium chloride, leucine, glycogen and cholesterol were most effective; then came potassium chloride and cholesterol plus one or another of the salts, and least effective were water and cholesterol in concentrations of from 1:100,000 to 1:1,000. The findings are discussed in the light of Leupold's speculations.

O. T. SCHULTZ.

MULTIPLE ADENOMAS IN AN UNDESCENDED TESTIS. N. IDE, Beitr. z. path. Anat. u. z. allg. Path. **91**:241, 1933.

Exploratory laparotomy in a man, aged 48, who complained of gastro-intestinal symptoms revealed multiple metastases in the omentum and mesentery. The primary tumor was not discovered. It was known that the man had an undescended right testis. Microscopic examination of one of the metastases led to the diagnosis of carcinoma probably originating in the gastro-intestinal tract. At necropsy some months later the primary tumor was found to be in the pancreas. The undescended testis, which was situated in the inguinal canal, was the size of an olive. On microscopic examination it was found to be atrophic but to contain multiple tubular benign adenomas of the type originally described by Pick.

O. T. SCHULTZ.

CONGENITAL NEUROFIBROMATOUS MACROGLOSSIA. H. MÜLLER, Centralbl. f. allg. Path. u. path. Anat. **57**:55, 1933.

This rare condition was found in the body of a boy 6 years old who was mentally retarded. Over the entire body there were numerous nevi, some finely

and some coarsely pigmented. There was also a tumor of the right side of the dorsal third of the tongue. In the tongue the papillae were enlarged, and scattered in the muscle were pinhead-sized nodules and strands of gray-yellow tissue. The nodules contained medullated nerves in their central parts but no ganglion cells. The muscle about them was loose.

GEORGE RUKSTINAT.

EPITHELIAL CYST OF THE HEART. A. KOLATSCHOW, Centralbl. f. allg. Path. u. path. Anat. **57**:311, 1933.

A smooth-topped cyst 21 by 18 by 17 mm. was found at autopsy on the inner part of the wall of the left ventricle of the heart of a man 45 years old. About four fifths of it projected above the endocardium. The content was jelly-like. The cyst wall was fibrous, lay near the muscle and had no inflammation about it; the lining was of cylindric epithelium, ciliated in some regions. The author found no reports of similar cysts in the literature. He believes that this occurrence is best explained as dystopia. Another possible origin would be from the myo-epicardial plate if the possibility of metaplasia of epicardial mesothelium into cylindric epithelium were granted. About ten years prior to his death the patient had had an echinococcic cyst removed from the liver, and during the operation the cyst broke, spilling its contents into the abdomen. The author sees no connection between the liver and a cardiac cyst.

GEORGE RUKSTINAT.

METASTASIZING GANGLIONEUROMA OF THE SYMPATHETIC SYSTEM. D. HEINRICI, Centralbl. f. allg. Path. u. path. Anat. **58**:1, 1933.

At the necropsy on a man 32 years old, who had an illness clinically resembling a disorder of the hematopoietic system, a tumor of the left abdominal sympathetic ganglion was found. The growth had two distinct portions, one of normal-appearing nerves and connective tissue and another of small round cells poor in cytoplasm. The latter cells occurred also in metastases in the ribs, vertebrae, liver and lumbar and peripancreatic lymph glands.

GEORGE RUKSTINAT.

SIGNIFICANCE OF THE SPLEEN IN THE GROWTH AND METABOLISM OF MALIGNANT TUMORS. W. BÜNGELER, Frankfurt. Ztschr. f. Path. **43**:409, 1932.

That the spleen may play an important inhibitory rôle in the development of tumors is suggested by the rarity of its involvement in such growths. From experiments with mice into which tumors were transplanted the author concludes that the spleen has an inhibitory effect on malignant growth. Extracts of splenic tissue inhibit fermentation and increase respiration of tumor cells but also, to a lesser degree, inhibit fermentation and increase respiration of normal cells. The inhibitory agent is water-soluble, is not bound to proteins or lipoids and is not destroyed by heat (100 C.) . The effects described are not confined to extracts of spleens but can be produced by extracts of other organs. These extracts, however, are less efficient.

O. SAPHIR.

DO GIANT CELL SARCOMAS EXIST? E. KOTZIAN, Frankfurt. Ztschr. f. Path. **43**:484, 1932.

The author reviews reported cases of giant cell tumors. While many such tumors are not malignant, not even being tumors in the sense of a neoplasm, the occurrence of true giant cell sarcoma cannot be denied. The author also discusses the various possible sources of the giant cells.

O. SAPHIR.

PRIMARY PLASMOCYTOMA. G. W. MOLOTKOFF, Frankfurt. Ztschr. f. Path. **43**:508, 1932.

Two cases are reported. In the first, the tumor was located in the submucosa of the appendix. It consisted almost exclusively of plasma cells with marked

pyroninophilic cytoplasm and eccentrically situated nuclei. Mitotic figures were present. A fine network of collagenous fibers was recognizable, sometimes in close relation to the cells. Occasionally giant cells were present. The tumor had invaded the muscularis and the mucosa. The author believes that this is the first instance of plasmocytoma of the appendix described in the literature. In the second instance the tumor was found in the neck of a 6 year old boy. The architecture of the involved nodes was obscured. There was a marked proliferation of plasma cells similar to those in the first case. Much reticulum was present. Occasionally giant cells with basophilic cytoplasm were found. The belief is expressed that in both cases the condition was a localized plasma cell lymphogranulomatosis.

O. SAPHIR.

EXTENSIVE BLASTOMATOSIS OF THE LYMPHOHEMATOPOIETIC APPARATUS. R. GLAUNER, Frankfurt. Ztschr. f. Path. **44**:105, 1932.

A 50 year old man had an enlargement of the axillary, inguinal and supraclavicular lymph nodes. One of the latter was removed and revealed a solid carcinoma. The patient died six months later, and autopsy disclosed small grayish-white foci in the pectoral, abdominal, psoas and iliac muscles and on the peritoneal surface. There were large, firm, white masses in the region of the lymph nodes corresponding to the bifurcation of the trachea. Small whitish nodules were found in the epicardium, the lungs, the spleen, the liver and the mucosa of the stomach and gastro-intestinal tract. The hepatogastric and hepatoduodenal ligaments also were diffusely infiltrated by whitish tumor masses. Tumor nodules were found in the cerebellum. The bone marrow corresponding to the right femur revealed large whitish areas surrounded by dark red bone marrow. The gross diagnosis was "disseminated blastomatosis." A primary tumor could not be found. Histologically the tumor nodes revealed a fine reticular and connective tissue network in the meshes of which there were round, oval and polygonal cells with large, well outlined nuclei showing fine chromatin network and distinct nucleoli. The cells were often well separated. Occasionally, however, they formed a syncytium. Sometimes giant cells, pyknotic nuclei and mitoses were present. Phagocytosis was frequentiy noted. Many cells contained lipoid granules. Occasionally nests of tumor cells were found in the lymphatics. The hypophysis revealed an adenoma consisting of chief cells. Because of the dividing of the tumor in organs belonging to the reticulo-endothelial system and because of the reticular structure of the tumor nodes, the marked phagocytosis of the cells and their ability to store fat, it is possible that this case is one of generalized disease of the reticulo-endothelial system (reticulosis). On the other hand, the fact that before death a node was removed which histologically revealed carcinoma and the fact that nests of tumor cells were found in lymphatics are in favor of the view that the nodules were metastases of a carcinoma, the primary carcinoma not having been found. The tumor formation might also be explained as a reaction of the reticulo-endothelial system to irritation either by the metastatic carcinoma cells or by their poisonous products.

O. SAPHIR.

COMBINATION TUMOR OF THE BRAIN (TRUE ANGIOGLIOMA). A. WEISS, Frankfurt. Ztschr. f. Path. **44**:144, 1932.

A tumor in the brain revealed structures of a cavernous hemangioma with true bone formation within a hyalinized stroma and those of a glioma which surrounded the hemangiomatous structures. The author believes that the hemangioma was the primary tumor, arising from a malformed anlage of the tuberculum intercolumnare. The anatomy and histology of this structure are discussed. It was first described by Putnam (*Bull. Johns Hopkins Hosp.* **33**:181, 1922). The opinion is expressed that the primary hemangioma caused a secondary glial reaction, which in turn formed the basis for the glioma. There also must have been a hereditary predisposition; otherwise a simple proliferation of glia would not have developed into a glioma.

O. SAPHIR.

FUNDAMENTAL QUESTIONS IN TUMOR RESEARCH. B. FISCHER-WASELS, Frankfurt. Ztschr. f. Path. **44**:177, 1932.

On the basis of the results of researches performed by himself and his assistants, the author refutes the theory that tumors have their origin in irritation of tissues, not because an irritation cannot be demonstrated but because too many factors are present all of which can be interpreted as irritation. Every factor must be analyzed separately and should not be disregarded or carelessly brought in consideration under the broad term of irritation. He does not believe that trauma alone is an important factor, and he contradicts a statement in the literature that a large number of tumors of the brain are caused by trauma resulting in primary areas of necrosis of the brain which produce reactive gliosis, which in turn gives rise to malignant growth. He maintains the importance of the "embryonal tumor anlage" and also of the hereditary factor. He summarizes a number of basic principles in regard to the formation of tumors, namely, "the law of primary local tissue insults," "the law of the typical latent period," which may vary from four to fifty-five years, "the law of the formation of a primary tumor anlage" and "the law of the sensible period of tumor formation." He also emphasizes the importance of regeneration in the formation of tumors. As to the question of "a special tumor constitution," he refers to experiments in animals which received tar for a long period. In these animals wounds as the result of burns did not heal with the formation of scars but underwent tumor formation. He concludes that tar and similar chemicals alter the constitution so that an irritant which under normal conditions results in a wound and scar may produce a tumor. A "tumor constitution" is produced also in producing alkalosis of the blood which is supposedly typical of cancer. The morphologic appearance of tumor cells, the marked irregularities of the nucleus and of the chromatin structures, and the many irregular mitoses make it seem possible that the active principles of the tumor lie in a changed nuclear substance of the tumor cells. Systematic examinations of the chemistry of the nuclear substances and the study of their metabolism by the use of the new methods of nuclein metabolism may offer a rich field for further investigation.

O. SAPHIR.

MULTIPLE CAVERNOMA OF THE PLEURA. G. MARTENS, Frankfurt. Ztschr. f. Path. **44**:272, 1932.

Multiple cavernoma was found in the pleura of a 56 year old man who died as the result of hemothorax. Microscopically the growths were true tumors. It is therefore not likely that they were remnants of healed pleuritis. The author discusses the relation between the hemorrhage into the chest and cirrhosis of the liver, which was also present.

O. SAPHIR.

TUMORS OF THE CHOROID PLEXUS, EPENDYMOMAS AND NEURO-EPITHELIOMAS. H. URBAN, Frankfurt. Ztschr. f. Path. **44**:277, 1932.

These tumors cannot be differentiated clinically, but they are distinguishable histologically. A hematoxylin-eosin-safranin method for studying their morphologic appearance is recommended.

AUTHOR'S SUMMARY.

SYSTEMIC RETICULO-ENDOTHELIAL HYPERPLASIA WITH TUMOR-LIKE FORMATIONS IN A CASE OF CHRONIC LYMPHATIC LEUKEMIA. J. LOESCH, Frankfurt. Ztschr. f. Path. **44**:351, 1932.

A 47 year old patient received antisyphilitic treatment for several years. About one year after the last treatment he noticed swelling of the axillary lymph nodes. He was admitted to the hospital with a diagnosis of chronic lymphatic leukemia. Autopsy revealed changes characteristic of lymphatic leukemia and also whitish nodes in the liver and spleen. Microscopically, a diffuse proliferation of reticuloendothelial cells was found in the spleen, liver and bone marrow. In the spleen

and liver this assumed the form of distinct nodules. Some of the lymph nodes showed changes characteristic of lymphatic leukemia, while others revealed marked proliferation of reticulum cells, which in some instances had completely replaced the lymphatic cells. Transitions from lymphatic cells to reticulum cells, however, could not be found.

AUTHOR'S SUMMARY.

Medicolegal Pathology

SUDDEN DEATH AND SYPHILIS. A. LEE BRISKMANN, Am. J. Syph. **16**:471, 1932.

Syphilitic disease is assigned as the cause in 30 per cent of twenty-four cases of sudden death from coronary sclerosis.

INJURY OF THE MEDULLA IN PUNCTURE OF THE CISTERNA MAGNA. A. R. VONDERAHE and F. C. HABERMAN, Arch. Neurol. & Psychiat. **29**:166, 1933.

A cisternal puncture was made in a patient for the diagnosis of meningitis. A second puncture was unsuccessful, and the patient died within four hours. Autopsy revealed two needle puncture wounds in the medullary portion of the brain stem with a surrounding hemorrhage that extended into the fourth ventricle.

WILLIAM FREEMAN.

CORONARY SCLEROSIS: AS SEEN IN A CORONER'S OFFICE. A. A. BERGER, California & West. Med. **36**:332, 1932.

The author analyzes statistically the causes of death in 4,754 autopsies that came to his attention from 1928 to 1930 inclusive. Approximately 50 per cent of the deaths were due to "natural causes" (nonintentional and nontraumatic). Approximately 8 per cent of all deaths due to natural causes (4 per cent of the total number of deaths) were due entirely to coronary disease in one form or another (ruptured heart, ruptured coronary aneurysms, coronary sclerosis with occlusion, coronary thrombosis). Coronary disease was found in 50 per cent of the remaining cases of death due to natural causes.

WILLIAM FREEMAN.

DEATH FOLLOWING INGESTION OF FIVE GRAINS OF ACETYLSALICYLIC ACID. B. R. DYSART, J. A. M. A. **101**:446, 1933.

A 45 year old white woman, unmarried, a teacher of art, who for fifteen years had suffered with increasing severity from "asthma" following an automobile accident, had taken epinephrine hypodermically for the terminal three years with only occasional relief. Allergically she was also sensitive to many foods. One day she took one 5 grain tablet of acetylsalicylic acid, and one minute later she went through an "asthmatic" attack and died before a physician could be called, the interval being probably about ten minutes. The postmortem examination by the coroner revealed only some tenacious mucus in the bronchi and a sarcoma of the dura about the size of an English walnut located over the left cerebral hemisphere.

WILLIAM FREEMAN.

BLOOD GROUPING IN QUESTIONS OF PARENTAGE. A. L. WIENER, J. Immunol. **24**:443, 1933; Am. J. M. Sc. **186**:257, 1933.

The chances of proving nonpaternity when the blood type of the falsely accused man is known were calculated. In New York City the average chances of proving nonpaternity by means of all four agglutinogens, A, B, M and N, are approximately one in three. A man of type A + + has less than one chance in ten to prove his innocence, and a man of type B + + has only one chance in seven. Men of the remaining ten types, however, have chances ranging from one in four

(O ++) to two in three (AB — +). The chances of detecting interchange of infants in hospitals by means of the agglutinogens A, B, M and N are seven in ten.

The medicolegal application of blood grouping for the determination of nonpaternity is urged on the basis of the experiences of European countries during the past ten years. The application of the agglutinogens M and N of Landsteiner and Levine is also fully justified at present on the basis of studies of families including more than 3,000 children. The method of application is illustrated by actual experiences in 6 cases, in 3 of which valuable information was obtained.

AUTHOR'S SUMMARIES.

TRAUMATIC RUPTURE OF THE PANCREAS. H. K. RANSOM, J. Michigan M. Soc. **31**:332, 1932.

The author reports a case of uncomplicated complete vertical rupture of the pancreas at the junction of the head and body in a 28 year old man who received severe abdominal injuries in a street fight.

WILLIAM FREEMAN.

SYMPOSIUM ON THE FORENSIC VALUE OF BLOOD GROUPING. M. Times **60**:203, 1932.

Attention is called to this symposium, in which George I. Swetlow discusses the legal applications of blood grouping; S. H. Polayes, the principles and the technic of determining the blood groups; A. S. Wiener, the theory of blood grouping with special reference to heredity, and Max Lederer, the practical application of blood grouping. The question of admitting the results of tests for blood grouping in courts in this country is discussed.

CHANGES IN THE SPLEEN AND MARROW IN ACUTE CARBON MONOXIDE POISONING. F. WIETHOLD, Deutsche Ztschr. f. d. ges. gerichtl. Med. **21**:325, 1933.

In a series of 200 fatal cases of acute carbon monoxide poisoning the gas was demonstrated in the blood of the spleen and the bone marrow. Increased hematopoiesis and vascular dilatation were observed in the marrow.

JACOB KLEIN.

PROTRACTED RETENTION OF ALCOHOL IN TRAUMATIC COMA. R. M. MAYER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **21**:337, 1933.

Alcohol previously imbibed may be found in the urinary bladder for a considerable time after death. In the author's case alcohol was demonstrated in the blood thirty hours after the onset of coma. Traumatic coma delays the excretion of alcohol.

JACOB KLEIN.

EXPERIMENTAL STUDIES ON POISONING BY INDUSTRIAL ARSENIURETED HYDROGEN. G. SCHRADER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **21**:342, 1933.

Observation of a fatal case of arsine poisoning in the industrial preparation of cadmium led to a more detailed histologic and experimental study. The outstanding lesions were hemolysis and degenerative changes in the kidneys, liver and heart. Experiments on guinea-pigs revealed similar changes, particularly in the liver and heart. It is of medicolegal interest to note that in some cases permanent cardiac damage may result.

JACOB KLEIN.

RUPTURE OF THE DUCTUS ARTERIOSUS. E. FRITZ, Deutsche Ztschr. f. d. ges. gerichtl. Med. **21**:365, 1933.

After an accident a woman gave birth to a fetus 43 cm. long which died four and one-half hours later. At autopsy on the fetus three small perforations of the

ductus Botalli were found. Microscopically these three areas showed complete tears with hemorrhage between the layers of the vessel wall and the adventitia. In explanation of this rare occurrence it is pointed out that hemorrhages of the vasa vasorum occur frequently in the ductus botalli. JACOB KLEIN.

FORENSIC SIGNIFICANCE OF BLOOD TYPES M AND N AND OTHER TYPES. F. SCHIFF, Deutsche Ztschr. f. d. ges. gerichtl. Med. **21**:404, 1933.

There are other blood types besides those indicated in the usual four group classification. By suitable tests with immune serum M and N types have been demonstrated. Moreover, group A of the ordinary classification is found to consist of two groups, A_1 and A_2. There is a P factor, as well as the factors excreted in the saliva. M and N are constant in their occurrence and follow the mendelian law. They are demonstrated even in the blood of the new-born. In medicolegal practice these groups are of value in studying cases of doubtful paternity, illegitimacy and exchange of infants, and even in identifying persons. Thus in a family of eight it was possible to distinguish between all but two by means of blood grouping.

JACOB KLEIN.

Technical

RELATION BETWEEN THE CEREBROSPINAL FLUID AND THE BLOOD SERUM IN SYPHILIS. F. E. HAAG and H. KOLBE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:380, 1932.

The extract of syphilitic liver, the citochol extract and the Bordet extracts were employed as antigens in the complement-fixation tests for blood serum and cerebrospinal fluid. The serums were also tested with three precipitation tests: the Meinicke, the citochol-Kahn and the Mueller *Ballung* reaction II. The cerebrospinal fluid was tested with the Lange colloidal gold test. In 1,400 cases, comparative tests of the blood serum and the cerebrospinal fluid were carried out. The precipitation tests were always specific; examination of the blood serum, particularly with precipitation tests, is essential for establishing the specificity of the results in examination of the cerebrospinal fluid, particularly when the latter shows a positive colloidal gold test with a negative complement-fixation reaction, as is frequently seen in multiple sclerosis. Positive signs in the cerebrospinal fluid with negative signs in the blood serum were occasionally encountered in persons with old treated syphilis of the central nervous system. Since the employment of the sensitive precipitation tests for the examination of the blood serum, the number of discrepant observations on the blood and cerebrospinal fluid has greatly diminished.

I. DAVIDSOHN.

DETERMINATION OF THE TITER OF ISO-AGGLUTININS. JORMA PIKKARAINEN and Y. K. SUOMINEN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:145, 1933.

The use of pipets (length, 30 cm.; volume, 0.1 cc.) for determination of the iso-agglutinin titer permitted a greater accuracy and uniformity than the use of test tubes, in a series of a hundred comparative tests. I. DAVIDSOHN.

QUICK DIAGNOSIS IN EPIDEMIC CEREBROSPINAL MENINGITIS. S. I. GINSBURG, W. S. KALININ and A. P. GULJAEWA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:463, 1933.

A complement-fixation test was developed by Ginsburg and his associates, employing as an antigen cerebrospinal fluid heated at 55 C. for thirty minutes. Polyvalent antimeningococcus serum served as an antibody. The performance of the test takes from three to four hours. Of 252 clinically established cases, 176 (70 per cent) were serologically, and 137 (54 per cent) bacteriologically, positive. In only 2 bacteriologically positive cases was the serologic test negative. In the

control series of cerebrospinal fluids, including those of persons with tuberculous and pneumococcic meningitis or cerebrospinal syphilis and normal persons, the test was negative. Control tests with the cerebrospinal fluid of patients having meningococcic meningitis were negative with normal horse serum and with antipneumococcus serum, while weak positive results were obtained when antigonococcus serum was used. Precipitation tests with cerebrospinal fluid and specific antimeningococcus serum showed a much lower degree of sensitivity than the complement-fixation tests. The positive reaction became negative after termination of the disease.

I. DAVIDSOHN.

COMPARISON OF THE RESULTS OF THE SACHS AND GEORGI (LENTOCHOL) REACTION AFTER TWENTY-FOUR AND FORTY-EIGHT HOURS. HEINZ ECKARDT, Ztschr. f. Immunitätsforsch. u. exper. Therap. **78**:497, 1933.

The test tubes were kept in the incubator for the first twenty-four hours and at room temperature for the second twenty-four hours. In 496 serums, or 6.5 per cent of the definitely syphilitic ones, only the second reading, after forty-eight hours, was positive. In 275 of the syphilitic serums these reactions were the only positive results, because the serums showed a negative Wassermann reaction. Nonspecific positive results were not observed. The results after forty-eight hours showed an increased sensitivity in cases of early syphilis, during provocative and during regular treatment. The inclusion of the reading after forty-eight hours raises the percentage of agreements with the Wassermann reaction to 96.3.

I. DAVIDSOHN.

PRECIPITATION TESTS FOR THE SEROLOGIC DIAGNOSIS OF SYPHILIS. Z. MILIŃSKA-SZWOJNICKA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **79**:139, 1933.

The citochol reaction I and II of Sachs-Witebsky, the "ball" reaction of Mueller and the Meinicke *Klärung* reaction were compared with the Bordet-Wassermann complement-fixation test, *27,273* clinically checked syphilitic serums and cerebrospinal fluids and 2,563 negative control serums being used. The citochol reactions showed the highest degree of sensitiveness and of specificity; the Meinicke test proved least satisfactory. Milińska-Szwojnicka warns against the replacement of the complement-fixation test with a precipitation test. Both should be employed simultaneously.

I. DAVIDSOHN.

THE PHYSICAL FITNESS OF RABBITS FOR THE PRODUCTION OF PRECIPITATING ANTISERUMS. P. VON GARA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **79**:171, 1933.

A total of 230 rabbits were employed for the production of precipitating antiserums in the Hygiene Institute of the University of Greifswald, which is one of the official serum institutes in Germany. The immunization was carried out according to Uhlenhuth. One cubic centimeter of serum, preferably fresh, was injected on the first, fourth and seventh days. Additional injections followed if the titer was less than 1:20,000. Not more than 15 per cent of the animals gave serums which were satisfactory in every respect, showing the necessity of using at least six animals in order to obtain a good specific precipitating serum. The best results were obtained with male white rabbits weighing over 2,500 Gm. During the summer, the serums showed fewer nonspecific heterologous precipitins.

I. DAVIDSOHN.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

E. H. HATTON, *President, in the Chair*

Regular Monthly Meeting, Dec. 11, 1933

THE ISO-ANTIGENIC PROPERTIES OF CASEIN. JULIAN H. LEWIS.

Casein is one of the several antigens of animal origin which lack species specificity; such an antigen from one species reacts in common with the same kind of antigen from other species. Chemically and immunologically casein from one source is identical with caseins from all other sources. Antigens which are indistinguishable immunologically should serve as antigen in the species from which they are obtained just as efficaciously as they do in foreign species. The iso-antigenic properties of crystalline lens, brain and testicle had been proved, but no test had, as yet, been made on casein.

A lactating goat was given sixteen intravenous injections of 1 Gm. of casein prepared from her own milk and dissolved at p_H 7. Another lactating goat was given similar injections of casein from cow's milk. The immune serums from these two goats, as well as the normal serums taken before the injections, were tested by complement fixation with caseins from cow's and from goat's milk. Both immune serums reacted equally with both types of caseins. This experimental proof of iso-antigenicity is also supported by clinical evidence. A lactating mother had allergic reactions when she weaned her child and attempted to stop the mammary secretion. Cutaneous tests made with her own milk gave severe reactions. Since the proteins of whey show strong species specificity and are incapable of exciting the formation of antibodies in the homologous species, it is probable that the active antigen in this mother was the casein.

DISCUSSION

H. C. SWEANY: What part does heredity play in allergy?

J. H. LEWIS: Heredity has a rôle in allergic diseases, but statistics vary as to its importance. A man subject to severe attacks of hay fever reared two children, one adopted, the other his own, under identical conditions. Hay fever developed in the son at 20 years of age but not in the adopted son.

SO-CALLED ALVEOLAR CELL CARCINOMA OF THE LUNG. H. C. SWEANY.

A uniformly low columnar cell carcinoma of the lung spread directly from one alveolus to another until all of the right lung and the middle of the left were consolidated masses with an ashen-gray appearance. The cells first supplanted the normal alveoli and maintained an alveolar arrangement. Some formed papillary growths into the alveoli. In the older region the cells disintegrated. Because of the low columnar type of cells the growth seems to have had its origin somewhere in the lower bronchi or bronchioles. There has never been proof that carcinoma of the lung has originated from the pulmonary alveolar cells. Probably the question can be answered only when the precursor of the alveolar cell is established.

DISCUSSION

V. LEVINE: I have seen several instances of carcinoma in the periphery of the lung. In some of the cases the growth originated in a dilated bronchus near the pleura. Thus, a carcinoma limited to the surface of the lung does not necessarily arise from alveolar epithelium.

CYTOLOGY OF NASAL POLYPS. THEODORE E. WALSH.

Interest has long been shown in the etiology of nasal polyps because of the differences in the clinical findings in the patients in whom they occur. Polyps occur in the nose as the result of a marked acute or chronic inflammation. However, it is not generally agreed that infection is necessarily the cause of the inflammation.

Clinically there are two groups of patients with nasal polyps: first, those in whom there is definite evidence of infection, as indicated by the presence of purulent secretion in the nose, and second, those in whom there is no exudate but rather a profuse watery nasal secretion, i. e., those with so-called vasomotor rhinitis. It has long been recognized by the more conservative observers that vasomotor rhinitis is not a surgical condition and that intranasal operative procedures in an uncomplicated case will not effect a cure, whereas when the inflammatory condition is due to sensitivity to some allergen, removal of the offending allergen will usually alleviate the symptoms and often effect a cure. On the other hand, a patient suffering from vasomotor rhinitis is usually subject to intranasal infection, and when infection is a complicating factor, operative interference is frequently a necessity. In the case of polyps, it is of value to know whether they are due to infection alone or are caused primarily by the vasomotor rhinitis, because on this knowledge depend the course of treatment adopted and the prognosis.

Some recent observations on the cellular components of nasal secretions in various cases of rhinitis led to the conclusion that in vasomotor rhinitis, whether allergy is demonstrable or not, the nasal secretions contain a high percentage of eosinophil leukocytes, whereas in infectious rhinitis there are never more than 5 per cent of eosinophil leukocytes in the secretions. This cytologic study was continued to include polyps in order to ascertain whether by correlation of the clinical findings and the cytology of the polyps any definite conclusion in regard to their etiology could be reached.

The polyps were fixed in a solution of formaldehyde or in Zenker's fluid to which a dilute solution of formaldehyde had been added, embedded in paraffin and cut at 10 microns. They were stained with Maximow's hematoxylin eosin azure stain. The cells were present in greatest number immediately beneath the basement membrane, while deeper in the tissue they were sparsely scattered. There appeared to be two distinct types of polyps—those with many eosinophil leukocytes and those with only occasional ones. In all the polyps examined there were numbers of mononuclear cells and many plasma cells. Polymorphonuclear neutrophils were seen in the polyps from persons who had clinical signs of infection. Of sixty persons whose polyps were examined, thirty-seven had polyps showing many eosinophil cells and twenty-three had polyps showing only an occasional eosinophil cell. Complete allergic investigations were made in nineteen of the thirty-seven persons whose polyps contained many eosinophil leukocytes and intradermal tests with one or more allergens were positive in fourteen and not in five of them. Of these five persons four had definitely low blood pressure and low rates of basal metabolism and one had marked hypertension. Of the twenty-three persons with few eosinophil leukocytes in the polyps, only two had complete allergic investigation and neither had any positive intradermal test or abnormality in blood pressure or metabolism. Of the thirty-seven persons with many eosinophil leukocytes in the polyps, fourteen had and twenty-three did not have clinical evidence of infection as shown by the presence or the complete absence of nasal exudate. However, of the twenty-three with few eosinophil leukocytes in the sections all had a purulent nasal discharge. In ten of these the pathologic conditions in the nose were unilateral and in thirteen bilateral, while in the thirty-seven who had many eosinophil leukocytes in the polyps, the pathologic changes were unilateral in only one and bilateral in thirty-six.

Diametrically opposite views on the etiology of nasal polyps have recently appeared in the literature; it is suggested on the one hand that allergy is to be

suspected in all cases of nasal polyps, and on the other that nasal polyps are always due to infection. The findings in the series of sixty cases reported here led to the following conclusions:

1. There are two types of nasal polyps, (*a*) those with many eosinophil leukocytes and (*b*) those with few.

2. Polyps with an abundance of eosinophil cells are due to the condition known as vasomotor rhinitis, which may be allergic.

3. Polyps with few eosinophil leukocytes are due to infection alone.

VASCULAR CHANGES IN CHRONIC EXPERIMENTAL ATELECTASIS. W. E. ADAMS.

The volume of the flow of blood in a normal lung and its variations under abnormal inflation have held the interest and attention of investigators in this field since the middle of the nineteenth century. Although extensive studies have been carried out by many investigators, no definite conclusion has been reached. The vascular changes occurring in an atelectatic lung during periods varying from a few days to over one and one-half years were studied.

Massive atelectasis of one lung was produced in forty-five dogs by completely occluding the bronchus to that lung by the technic in which nitrate of silver is used (*Ann. Surg.* **95**:106, 1932). The animals were killed at intervals of from a few days to one and one-half years. India ink was injected into the blood vessels in a portion of the atelectatic lung in some of the animals, according to the technic of Coryllos and Birnbaum (*Arch Surg.* **19**:1346, 1929). In order to obtain microscopic sections of atelectatic tissue in the same lung before and after the injection of india ink, a portion of the lung was ligated through a thoracotomic opening before the injection. In another group of animals, reinflation of the atelectatic tissue was accomplished through a thoracotomic opening by injecting air through a needle inserted into the bronchus distal to the region of stenosis. Reinflation was obtained at a pressure of between 35 and 40 mm. of mercury. Again a portion of the reinflated tissue was tied off before the injection of india ink. Thus microscopic sections of reinflated atelectatic tissue before and after the injection of ink were made available.

Microscopic sections of pulmonary tumors that had been atelectatic for a few days revealed dilated and tortuous capillaries readily filled with india ink. The capillaries were so numerous that the tissue resembled hemangioma. Atelectatic lobes at this stage could be reinflated to almost their original size. By the end of one month the number and size of the capillaries in the atelectatic tissue had decreased. There were no other changes. By the end of four months there were large spaces filled with blood, chiefly at the periphery of the parenchyma of the lung. These appeared to be alveoli containing blood. At this stage a considerable amount of fragmentation and desquamation of bronchial and alveolar epithelium was noted. From the fourth until the fifteenth month there was a gradual increase in the size and number of the blood spaces with a decrease in the number of alveolar walls. A gradual increase in the amount of tissue with changes was also observed. Not infrequently there was blood in the smaller bronchioles. Following the injection of india ink there appeared to be a partial washing out of the blood from the spaces, but at no time was ink observed within them. Reinflation of atelectatic tissue during this period caused a moderate tearing of the alveoli. The introduced air entered only a portion of the spaces filled with blood. There appeared to be less blood removed from the spaces following reinflation of the atelectatic tissue.

From these observations it is difficult to understand how the blood in the spaces could have been supplied by the pulmonary circulation. The lack of blood pigment in any of the microscopic sections precludes the possibility that the blood in the spaces was a hemorrhage into the pulmonary parenchyma. That some of the spaces contained less blood following the injection of india ink, together with the lack of blood pigment, strongly suggests that this blood was in the circulation. Possibly the spaces filled with blood were in direct connection with the bronchial

arterial system and the pressure in this system may have been sufficiently great to break through an atrophied and degenerated wall between the original alveoli and the circulating blood. Evidence of degeneration and fragmentation of the epithelium of both the bronchioles and the alveolar walls lends support to this view. If this is true, injection through the pulmonary system would be less likely to replace the blood in such spaces with india ink. This question is being investigated.

UNUSUAL PEPTIC ULCERS OF THE GREATER CURVATURE OF THE STOMACH. WARREN B. MATTHEWS.

A woman aged 57 had had symptoms of ulcer without complications for thirty-five years. Roentgen examination demonstrated a filling defect of the most dependent portion of the greater curvature of the stomach. No free acidity was obtained in an Ewald test meal. Two thirds of the stomach was resected by Dr. Phemister. The patient has been followed for five and one-half years since the operation. She has remained in good health. The excised tissues contained an ulcer 1.5 cm. in diameter astride the greater curvature 12 cm. from the pylorus. The ulcer had perforated only to the muscularis. Microscopically it was a typical chronic peptic ulcer, without malignant changes.

A man aged 56 had suffered epigastric pain for one year. In spite of a loss in weight of 20 pounds (9 Kg.) he seemed in fair health. Roentgen examination revealed a large crater in the middle third of the greater curvature of the stomach. An injection of histamine stimulated a gastric secretion with a free acidity of 52 clinical units. The preoperative diagnosis was: carcinoma. Dr. Phemister removed two thirds of the stomach because of a lesion on the greater curvature, which was adherent to the transverse colon. The patient has been well for fifteen months since the operation. The excised mass had an ulcer overriding the greater curvature, 15 cm. from the pylorus, which had perforated all the layers of the wall of the stomach and had a base of thickened, indurated omentum. Microscopically the lesion was a scarred peptic ulcer considerably infiltrated at the base by round and plasma cells and eosinophil leukocytes.

The reports in the literature on this subject are unsatisfactory because of the incomplete and confusing data. About 22 reports of peptic ulcer situated exactly on the greater curvature apart from the pylorus were found in which the condition was described more or less accurately. Histologic examinations were mentioned in reference to 16 of these 22 cases. On the other hand, mention was made of 140 cases of the type concerned here in reports of 4,380 cases of peptic ulcer affecting all parts of the stomach. These 140 cases were mentioned only incidentally; the ulcers were not described and, so far as the reports indicate, were not studied histologically.

DISCUSSION

DALLAS B. PHEMISTER: I have no doubt that operations have been performed on a great number of patients with the mistaken diagnosis of carcinoma, as in these cases. In the second patient I suspected a perforating ulcer. There had been no infiltration of the entire stomach.

Book Reviews

An Outline of Immunity. By W. W. C. Topley, M.A., M.D., F.R.C.P., F.R.S., Professor of Bacteriology and Immunology, University of London. Price, $6. Pp. 415, with illustrations. Baltimore: William Wood & Company, 1933.

The substance of a great part of the material in this book appears in Topley and Wilson's "Principles of Bacteriology and Immunity," which was reviewed in the ARCHIVES (**9**:1306 [June] 1930). "It has, however, been entirely rewritten. In some places it has been compressed, in others extensive additions have been made." The question of the need or desirability of this separate elaboration of the part on immunity in the earlier book is answered by the author's plea for more attention in the medical curriculum to the great advances in immunity, no matter what department may be entrusted with its teaching. On account of its practical importance, the fundamental principles should be taught in the preclinical years so that the student may be prepared for their applications to clinical medicine a little later. The prospective physician should have a basis of sound knowledge on which to develop his judgments on immunologic procedures in practice. Immunity is an applied science that enables the physician and the epidemiologist to intervene advantageously in the course of infectious diseases. It is from this point of view that the book has been written. In his introduction the author discusses the general relations of immunity to epidemiology and to clinical medicine, leading up to the consideration in the second chapter of the important and difficult matter of accurate measurement of immune conditions in the living. The need of sound statistical methods in immunologic work is made clear at the very beginning. A glance at each succeeding chapter, if only to note its title, will indicate the scope and general plan of the book. Chapters 3 and 4 deal with the mechanisms of bacterial infection and the mechanisms that hinder or prevent the access of bacteria to the tissues. The next six chapters are concerned with antigens and antibodies, their nature and their reactions; the antigenic structure of bacteria, knowledge of which has advanced rapidly in recent years; the mechanisms of antitoxic and of specific antibacterial immunity; the response of bacteria to the defenses of the host; the origin of the natural antibodies and their rôle in specific immunity, and the antibody-forming apparatus and its reactions. Chapter 12 reviews anaphylaxis, hypersensitiveness and allergy. As an example of the author's way of summarizing the evidence presented, the concluding paragraph of the summary at the end of this chapter is given in full:

"Acute anaphylaxis, natural hypersensitiveness, the allergic response and specific antibacterial immunity would appear to depend on the same fundamental mechanism —the union of an antigen with its specific antibody. The differences between them depend on the nature of the antigen concerned (living parasite, toxic substance or intrinsically inert material), the nature of the animal into whose tissues it penetrates, the rate and route of penetration, the distribution of the antibody in the animal's tissues and body fluids, the secondary effects of the primary cellular reactions, and a variety of other factors."

The three next chapters discuss nonspecific mechanisms in general immunity, local immunity and the influence of diet, fatigue, changes in temperature and humidity, chemical agents and other factors on immunity. The review of immunity in virus diseases (chapter 16) leads to the conclusion that there is no fundamental distinction between antivirus and antibacterial immunity. It is suggested that the intracellular confinement of viruses may play a rôle in the greater effectiveness of antivirus immunity. Then comes a chapter on herd infection and herd immunity, a complex process the understanding of which has been furthered by the investigations of the author himself. The concluding parts of the book are devoted to the

practical application of immunity in diagnosis (in the diagnosis of infection, of herd infection and of immunity), in prophylaxis (antitoxic, antibacterial and antivirus immunization) and in treatment. The final chapter deals with the standardization of immunologic reagents.

This hurried account of the contents reveals the wide scope of the book. All phases of immunity, the scientific as well as the applied, are considered. The general plan is to set down the evidence fully and fairly and then to draw the conclusions that appear to be warranted. By means of different type forms the author indicates his ideas of the relative importance of the various parts of the text. Each chapter has a list of references, more copious than usual, so that those who are "wise enough to prefer their own conclusions to those offered by others may find access to the records." The style is clear and fluent. The author is the critical master of his subject in practically all parts. This does not mean that he may not be wrong in some of his conclusions, as, for instance, that there is only one specific streptococcus toxin. It is safe to say that no book written in English presents immunity in all its aspects so comprehensively and adequately as Topley's, but it goes over the head of the average medical student in the preclinical years. For the advanced student, the special worker and the teacher, however, there is no better book on immunity available at this time.

Histology. By S. Ramón y Cajal, M.D. (Madrid), F.R.S. (London), LL.D. (Clarke); Director, Royal Cajal Institute for Medical Research; Emeritus Professor of Pathology, University of Madrid Faculty of Medicine; Nobel Premiate in Medicine; Life Senator of Spain. Revised by J. F. Tello-Múñoz, M.D. (Madrid), Professor of Pathology, University of Madrid Faculty of Medicine. Authorized translation from the tenth Spanish edition by M. Fernán-Núñez, M.D. (Madrid), Professor of Pathology, Marquette University School of Medicine. Price, $8. Pp. 738, with 535 illustrations. Baltimore: William Wood & Company, 1933.

From the translator's preface: "The present work is a translation of the elementary student's textbook of Prof. R. S. Ramón-Cajal, dean of Spanish histologists, for the purpose of presenting in English a compendium of the investigations of that author and his disciples." As was to be expected, the outstanding part of this book is the section devoted to the nerve tissue. This contains a summary of Cajal's views and many of his classic figures, and is further embellished by Tello-Múñoz' excellent illustrations of nerve endings. The other primary tissues are treated in some detail, but the microscopic anatomy of the organs is treated very briefly and in places the information is inadequate and out of date. For example, one would like to see more than ten lines devoted to the islands of Langerhans. A valuable feature of the book is the illustrations of the Golgi network in many unusual types of cells.

There are some points in the book which merit unfavorable criticism. According to the translator's preface, "the English version is an almost exact literal translation of the Spanish text, so that the nomenclature, phraseology and syntax will often appear unusual. This has been done with a view to rigid accuracy in translation, and the desire to enrich our histologic nomenclature by coining into English many of the vivid teaching locutions which made Professor Cajal's lecture room a Mecca for students." The reviewer cannot sympathize with the translator in his intention of enriching the English histologic nomenclature. The teacher and student of histology are sufficiently burdened with the present English terminology and the need for carrying the French and German equivalents. An unexpected disadvantage of this new terminology is the fact that the index is often of little aid in using the book, for it does not contain many of the common histologic terms (since these are not used in the text). The reviewer also objects to a translation so rigorous that Meves is spelled "Mevez" and Tomes "Tomez." The "almost exact literal translation" is incomprehensible in some places and inaccurate in others, as on page 30, the "Malpighian corpuscles of the skin." An accurate but freer translation would have made the text more easily understood.

There are many typographic errors, some of which are inexplicable, as, on page 30, the occurrence of "neutrophilic" and "eosinophylic" in the same line. Some of the figures taken from other authors are not credited to them, as figure 182 (von Ebner) and figure 189 (von Korff); in a few cases the figures have been credited in the text but not in a legend accompanying the illustration.

It is not fair to omit Altmann's name from the discussion of mitochondria and to credit the discovery to Benda. It is probable that most cytologists would disagree with the statement on page 16: "Concerning the function of the mitochondria most cytologists see in them the basic material entering into the formation of individual cells and serving as a basis of protoplasmic inheritance."

To the extent that the book calls attention to the works of the Spanish histologists, it is an addition to the English histologic literature. But the reviewer wonders whether many of the references to Cajal's investigations of forty years ago represent his views today—as, for instance, the idea expressed on page 609 regarding the hypophysis: "Cajal's investigations (1894) showed that the epithelium of the intermediary portion possesses the character of a sensory organ, since, besides containing certain cells, which can be considered homologous to the sensory epithelia of the organ of Corti, are also found arborizations of free nerves, terminating among the cells of the epithelial covering and proceeding from fibers arising in the nervous lobule."

Cajal's "Degeneration and Regeneration in the Nervous System" was translated into English in 1929. His "Histologie du système nerveux de l'homme et des vertébrés" is out of print. Perhaps a greater service would have been rendered English-speaking anatomists had this or other classic writings of Cajal been translated, rather than his elementary "Histology."

Number 4 Canadian Hospital. The Letters of Professor J. J. Mackenzie from the Salonika Front. With a Memoir by his Wife, Kathleen Cuffe Mackenzie. Price, $2.50. Pp. 247, with 13 illustrations. Toronto: The Macmillan Company of Canada, Ltd., 1933.

Beginning with well written biographic notes, in which Mackenzie's descent from a distinguished Scottish line and his professional training under such men as Ramsey Wright, His, Braune, Karl Ludwig and Koch are outlined, there follow comments on his early career as professor of pathology at the University of Toronto. Many honors were conferred on him, including the presidency of the American Association of Pathologists and Bacteriologists (1914). His work, his inclination to sound problems of research and his interest in art, music and literature are interwoven in a philosophic vein, with an outlook on life and its meanings which will excite the sympathy of those who are closely bound by ties of deepest affection. The introduction merges into the description of the departure of Number 4 Canadian Hospital for its tour of duty abroad; then follow the letters from Salonika, which begin on Oct. 22, 1915, and continue practically daily to June 29, 1916. Mackenzie went back to London, where his wife had been waiting, and after a period of investigative work with Brodie returned to Toronto and his professional duties. The book closes with a brief summary of his life thereafter until his death in 1922 from streptococcic endocarditis.

The nature of the work of a scientist and the necessarily objective character of his scientific publications sometimes serve to conceal the traits of his personality even from those who have frequent professional contacts with him. The academic career is often pursued by men who are shy, and the seclusion in which much of their work is done does nothing to overcome this inherent reserve; and this was true of Mackenzie. With him, as with others of his kind, the quiet atmosphere of the writing desk served to unleash diffidence and to reveal the man himself. The letters are written in the simple and clear English of a person with a fine background, cultural refinement and a broad education. An undercurrent of love and devotion illuminates without dominating the more prosaic but deeply interesting account of the activities of the Hospital, the postmortem examinations, the bacterio-

logic problems, the journeys into the neighborhood, the critique of things political and military and the analytic comments on the extensive reading that could be done in those periods of quiet which intervene in the military hospital between the times of strenuous activity.

During the course of the World War the medical work of the armies was carried on in large part by those who, like Mackenzie, left pressing civilian duties and went overseas to labor in field and hospital. To all those this book will be a source of interest, enjoyment and appreciation. Most of the newer generation of doctors and nurses were too young in those stirring days to serve. To them the book will be instructive in its depiction of war without diagrams and maps of military actions, without figures as to divisions and regiments, without numbers of casualties and deaths, but war as it is seen in those tragic times when the sick and wounded are returned back of the lines for suffering and often death, for invalidism and for reconstruction. In this recountal the younger reader will find that valor in the face of dangers, seen and unseen, so conspicuous among the troops, was shared in no inconsiderable measure by the doctors and nurses and other members of the medical services of the armies. The book is almost devoid of technical terms and can be recommended also to the lay reader.

Les troubles de l'élimination urinaire de l'eau. Etude physiopathologique et clinique. By Jules Cottet, Ancien interne des hôpitaux de Paris. Price, 32 francs. Pp. 212. Paris: Masson & Cie, 1933.

Water is an important constituent of protoplasm and plays an active part in its metabolism. Cottet reports observations on tests of renal function, renal physiology, prerenal water metabolism and his clinical experiences with the water cure or provoked diuresis. He noted that the metabolism of water may be disturbed by abnormalities in absorption from the gastro-intestinal tract; by circulatory changes, especially cardiac insufficiency, and by excessive elimination through extrarenal pathways. These factors may be combined in infinite variety under clinical conditions. The normal kidney concentrates or dilutes the urine according to need, while the diseased kidney exhibits no functional rhythm but excretes urine of a fixed low specific gravity. Clinically, it is necessary to estimate in each case the renal secretory and diluting power, as well as the extrarenal factors affecting water metabolism. For this purpose the author studied the rhythm, quantity and specific gravity of the twenty-four hour urinary output of a patient who for five days previously had taken 5 Gm. of salt daily to establish an equilibrium between chlorides and water. The urine was collected and studied in three fractions: (1) a night specimen, from 9 p. m. to 7 a. m.; (2) a morning specimen, from 7 to 9 a. m., and (3) a day specimen from 9 a. m. to 9 p. m. The urine was measured; the color and specific gravity were recorded, and the amount of chlorides and urea determined. Also determinations of blood urea were made and compared with the urinary excretion of urea. Diuresis was provoked in the morning specimen by having the patient drink 600 cc. of water between 6:30 and 7:00 a. m. Normally a diuresis results which frequently exceeds the amount of water ingested. The amount of day urine was normally three times as great as that of night urine. With insufficiency of renal secretion there were absence or weakness of provoked diuresis, absence of orthostatic oliguria, an increase in night urine and fixation of density at a low level. With prerenal abnormalities in water metabolism there were marked orthostatic oliguria, excessive polyuria in the reclining position, equality in the amount of night and day urine and dissociation in the elimination of water and chlorides, that is, nocturnal polyuria, associated with diurnal polychloruria. The author describes and criticizes other functional tests, such as those of Violle, Bergouignan, Vollhard and Mosenthal. He discusses nycturia, orthostatic oliguria, urinary density, the rhythm of the excretion of chlorides and their clinical correlation with gastro-intestinal, hepatic, cardiovascular and renal disorders. Thus, he shows that cardiac insufficiency is characterized by marked oliguria, with uniformity of urinary volume and density. Urinary insufficiency may also be due to defective renal secretion, to retention of water and

chlorides or to oliguria of extrarenal causation. He describes mild types of oliguria and retention of urea due to a habitually inadequate intake of water. He cautions that in the therapeutic application of water in provoking diuresis, it is necessary to adapt the cure to the clinical condition of the patient, with particular regard to conditions of absorption, circulation and renal secretion. The book is written by a clinician; it is based on clinical studies and is chiefly for practitioners. As such it should be of value to all physicians interested in renal function and water metabolism.

Physical Chemistry of Living Tissues and Life Processes as Studied by Artificial Imitation of Their Single Phases. By R. Beutner, M.D., Ph.D., Professor of Pharmacology, School of Medicine, University of Louisville. Price, $5. Pp. 337, with 79 illustrations. Baltimore: Williams & Wilkins Company, 1933.

This volume deserves a wider sale and reading than it will get—the former because the publisher has hazarded investment on a monograph, and the latter because it is concerned with cellular physiology, which is at once the most difficult and important phase of modern biologic study and the most "theoretical." Beutner makes clear the problem in his opening sentences. The purpose of physiology is to explain the dynamics of a machine and the anatomic, chemical and physiologic analyses of the living machine are insufficient to this end. The physiologist or pathologist must therefore construct from the nonliving materials of the universe new machines (or models, as Beutner calls them) which "work" as does the living organism and by such "synthesis" build up systems that qualitatively and quantitatively are the analogs of living matter. Toward such an end Beutner makes three "attempts at approach," discussing in the first, membranes and osmosis, in the second, crystallization and surface forces, and in the third, the electrical changes observed in living matter. The third is peculiarly that of the author (phase boundary potentials), though even in this he brings under discussion the related labors of other men, such as those of Lapicque on "chronaxie," of Ralph S. Lillie on wave transmission in iron wire and of Osterhout on electrical conduction in injured tissues. He is even more generous in discussing the findings and opinions of other authors in the remaining half of the volume. For this reason the modern biologic worker perhaps cannot be referred to a better volume than this for orientation on the mechanics of growth, the rôle of osmotic pressure, "membrane equilibrium," the function of lipoids, colloid swelling (very weak), intracellular oxidation, cellular "permeability," emulsions, protoplasmic structure, "micelles," solid and liquid crystals, surface tension and adsorption. Beutner thus demonstrates how in the nonliving world the most various types of systems are known which, without recourse to meaningless "vital" explanations, give a clew to what may be happening in the living. But he attempts little critique of mutually exclusive findings or opinions and makes no effort to coerce the reader into any one pathway of biologic thought. The synthesis of what living matter may be after all is therefore largely left to the reader himself, and he is repeatedly warned against premature conclusion by Beutner's constantly reiterated, "But of this matter we know nothing."

L'infection chez les insectes; immunité et symbiose. By A. Paillot, Docteur ès-sciences, Lauréat de l'Institut; Directeur de la Station de Zoologie Agricole du Sud-Est. Cloth. Price, 100 francs. Pp. 535, with 279 figures. Lyon: Librairie Médicale et Scientifique, 1933.

The scope and general nature of this interesting book can be illustrated by briefly indicating its subdivisions. There are seven parts. The first four deal with infections of insects by protozoa, fungi, ultraviruses and bacteria, respectively. The fifth part is devoted to the consideration of natural and acquired antibacterial immunity in invertebrates. In the sixth part the author discusses symbiosis in the plant louse (*puceron*). In the seventh part certain economic

considerations of infections in insects and the rôle of insects and other arthropods in the transmission of infections are discussed. No attempt will be made to review the contents in detail. The presentation is clear, direct, orderly. The book is well printed and well illustrated. Undoubtedly, the study of the diseases of insects due to ultraviruses is bringing to light new facts that may contribute to a better understanding of these diseases in general. Antibacterial immunity in invertebrates appears to be associated in a large degree with humoral phenomena, and phagocytosis seems to play only an insignificant rôle. In certain insects symbiosis may be regarded as natural antibacterial immunity. In view of the interest in the rôle of insects in transmitting infectious diseases to man, animals and plants, Paillot's book will be heartily welcomed. It is an important and stimulating contribution to the comparative pathology of infectious diseases.

The Anatomy of the Rhesus Monkey (Macaca Mulatta). By T. H. Bast and others. Edited by Carl G. Hartman, Department of Embryology, Carnegie Institution of Washington, and William L. Straus, Jr., Department of Anatomy, Johns Hopkins University, Baltimore. Cloth. Price, $6. Pp. 383, with 128 illustrations. Baltimore: Williams & Wilkins Company, 1933.

This book has been produced by the joint efforts of interested scientists to meet the growing need for a handbook on the anatomy of the rhesus monkey. It is the first comprehensive work on the anatomy of an Old World monkey. Each of the eighteen chapters has been written by an especially qualified author or authors actively engaged in research closely related to the subject in question. There is an appendix on the housing and care of monkeys. The illustrations, almost all of which are drawings by Benjamin Kopel, are excellent. At the end of each chapter is a selected bibliography. The terminology of human anatomy (Basle anatomic nomenclature) is used. There is no apparent reason for disagreeing with the editors in their statement that "the book attains the immediate object of affording a useful description of the anatomy of the rhesus monkey for research workers as well as for such classes in comparative anatomy as contemplate using this animal as the object of dissection."

Atlas of Pathological Anatomy. Volume I. Compiled by E. K. Martin, M.S., F.R.C.S. Issued under the direction of the editorial committee of the British Journal of Surgery. Price, $15. Pp. 489. Baltimore: William Wood & Company, 1933.

This atlas contains the drawings of pathologic specimens published in the supplement to the *British Journal of Surgery* during the five year period ending with 1930. The object of the compilation is to foster a greater interest by surgeons in morbid anatomy, especially as related to clinical surgery. The supply of suitable material for this purpose is unlimited. The specimens illustrated in the volume are from the great Hunterian Museum of the Royal College of Surgeons and, to a less extent, from museums of medical schools. The volume covers tumors of bone, diseases of the stomach, breast, kidneys and gallbladder and bile ducts and inflammations of bone. Not rare conditions, but typical examples of well known diseases, are represented. The text consists of brief descriptions of the gross and microscopic material, followed by a brief statement of the clinical features. There is no statement as to the preparation of the microscopic sections. The pictorial part presents a large number of good colored illustrations of gross specimens and also black and white drawings of gross specimens and typical microscopic fields. There are reproductions of a few roentgenograms. Generally speaking, all the reproductions are excellent. The microscopic drawings in some cases are perhaps too diagrammatic. It is planned to continue the publication until it will include practically all subjects that can be illustrated with museum specimens. Undoubtedly, the atlas will help to interest the surgeon in a closer study of the structural characteristics of the conditions that come under his hand and eye.

The Biology of Bacteria: an Introduction to General Microbiology. By Arthur T. Henrici, M.D., Professor of Bacteriology, University of Minnesota. Price, $3.60. Pp. 472, with 112 figures. Boston: D. C. Heath & Company, 1934.

The preface states that "this is a textbook to be used by general or nontechnical students who will take but one course in microbiology; or in introductive courses for other students who will be taught particular applications of the science in advanced courses." The book, then, stresses fundamental knowledge of the subject rather than application. As indicated by the title, it deals for the most part with bacteria, but other microbes also receive proper consideration. The first sixteen chapters deal with the history of bacteriology; the microscope; a survey of microbic life (protozoa, algae, fungi, bacteria and ultramicrobes); the finer structure, growth and reproduction, heredity and variation, metabolism, cultivation and death of bacteria, and infection and immunity, including bacterial diseases of plants. Then comes a chapter on the classification of bacteria, which is followed by chapters on Nitrobacteriaceae, Coccaceae, Spirillaceae, Bacteriaceae, Bacillaceae, Mycobacteriaceae and Actinomycetaceae. As indicated, the classification of the American Society of Bacteriologists (Bergey's Manual) is followed. The final chapters are devoted to molds, yeasts, pathogenic protozoa, Spirochaetales, Rickettsiae and virus diseases. At the end of each chapter are brief suggestions for further reading. The illustrations, original and borrowed, are good. The style is clear and pleasant. The book is recommendable without reservation as a sound and useful textbook in its field.

CORRECTION

An error occurred in the last sentence of the abstract of the article by C. V. Harrison entitled "Arterial Disease Produced by Cholesterol and Vitamin D" in the January issue. This sentence should read: "It is concluded that the movements of the vessel determine the localization of both vitamin D and cholesterol lesions and probably also human arterial disease."

Books Received

PRÉCIS DE MICROSCOPIE. M. Langeron, Chef de laboratoire à la Faculté de Médecine de Paris; Chef des travaux de parasitologie à l'Institut de médecine coloniale. Cinquième édition, revue et augmentée. Paper, 86 francs; cloth, 100 francs. Pp. 1205, with 355 figures. Paris: Masson et Cie, 1934.

THE BIOLOGY OF BACTERIA: AN INTRODUCTION TO GENERAL MICROBIOLOGY. Arthur T. Henrici, M.D., Professor of Bacteriology, University of Minnesota. Price, $3.60. Pp. 472, with 112 figures. Boston: D. C. Heath & Co., 1934.

THE THYROID GLAND: ITS CHEMISTRY AND PHYSIOLOGY. Charles Robert Harington, M.A., Ph.D., F.R.S., Professor of Pathological Chemistry in the University of London. Pp. 222, with 28 figures. London: Oxford University Press, 1933.

ANNUAL REPORT OF THE INSTITUTE FOR MEDICAL RESEARCH FOR THE YEAR 1932. A. Neave Kingsbury, Director, Institute for Medical Research, F.M.S., Kuala Lumpur. Pp. 108. Kuala Lumpur: Federated Malay States Government Press, 1933.

PUBLICATIONS OF THE DEPARTMENT OF PATHOLOGICAL ANATOMY AND HISTOLOGY OF THE HUNGARIAN ROYAL FRANCIS JOSEPH UNIVERSITY, SZEGED, HUNGARY. Volume 3. Edited by Josef Baló. Szeged, 1933.

LES TUMEURS BÉNIGNES DU LARYNX: ÉTUDE ANATOMIQUE ET CLINIQUE SUIVIE D'UNE BIOGRAPHIE DE L'AUTEUR. Professeur Ricardo Botey. No. 25, Monographies oto-rhino-laryngologiques internationales. Publiées par M. Vernet, L. Ledoux, G. Pôrtmann, H. Aloin et M. Sourdille. In memoriam. Paper. Price, 25 francs. Pp. 79, with 26 illustrations. Paris: Les Presses Universitaires de France, 1932.

L'ETHMOÏDITE. Patrick Watson-Williams et Eric Watson-Williams. No. 22 Monographies oto-rhino-laryngologiques internationales. Publiées par M. Vernet, L. Ledoux, G. Portmann, H. Aloin et M. Sourdille. Analyse en anglais. Paper. Price, 35 francs. Pp. 144, with illustrations. Paris: Les Presses Universitaires de France, 1932.

A STUDY OF GROWTH AND DEVELOPMENT: OBSERVATIONS IN SUCCESSIVE YEARS ON THE SAME CHILDREN. R. M. Fleming, with a Statistical Analysis by W. J. Martin. Medical Research Council, Special Report Series, No. 190. Price, 1s. 6d. Pp. 85. London: His Majesty's Stationery Office, 1933.

THE LYOPHILIC COLLOIDS (THEIR THEORY AND PRACTICE). Martin H. Fischer, Professor of Physiology in the University of Cincinnati, and Marian O. Hooker, Research Associate in Physiology in the University of Cincinnati. Price, $4.50. Pp. 250, with 84 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1934.

NEUROLOGY. Roy R. Grinker, M.D., Associate Professor of Neurology, University of Chicago. Price, $8.50. Pp. 974, with 401 illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1934.

DIET AND THE TEETH. AN EXPERIMENTAL STUDY: PART III. THE EFFECT OF DIET ON DENTAL STRUCTURE AND DISEASE IN MAN. May Mellanby. Medical Research Council, Special Report Series, No. 191. Price, 5s. Pp. 180, with 46 plates. London: His Majesty's Stationery Office, 1934.

A DIABETIC MANUAL FOR THE MUTUAL USE OF DOCTOR AND PATIENT. Elliott P. Joslin, M.D., Clinical Professor of Medicine, Harvard Medical School; Medical Director, George F. Baker Clinic for Chronic Disease at the New England Deaconess Hospital; Consulting Physician, Boston City Hospital, Boston. Edition 5, thoroughly revised. Price, $2. Pp. 224, illustrated. Philadelphia: Lea & Febiger, 1934.